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Presentedby:

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Postprandial glucose and lipids metabolism state among obese patients with type 2 diabetes

Discussed on:

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To

My Family

My Teachers

Foreword

This PhD thesis is interested in studying the postprandial versus fasting states as controversial issues in obese patients with type 2 diabetes, comparing to two control groups, in the north-western region of Algeria. The study is based on research work focusing on anthropometric, nutritional and biochemical parameters. The manuscript is structured as follows:

- The first section describes theoretical considerations about obesity (Chapter 1), type 2 diabetes (Chapter 2) and the metabolism of the postprandial states (Chapter 3);
- The second part, covers the study design and the performed protocol according to the preliminary hypothesis and objectives of the study (Chapter 4);
- The third section concerns the results of the study (Chapter 5);
- And the conclusion which draws recommendations that might help researchers and health care professionals to manage correctly the postprandial state among people with type 2 diabetes.

The wording of this manuscript follows the latest version of ISO-690 (*International Organization for Standardization*) standard for bibliographic referencing in documents preparation, content, form and structure, published in 2010.

The research work in connection with this thesis has involved a great number of people, whom I am indebted to and I wish to thank them all for their help and support.

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Abstract

Background: The magnitude of issues related to healthcare among type 2 diabetes patients is the result of the disease itself and also its association with several risk factors for cardiovascular diseases such as overweight, obesity and dyslipidemia. The overall goal of health behaviour and therapy interventions in people with diabetes who are overweight or obese is based on metabolic risk assessments that are measured by referring to fasting recommendations. However, it has been recently shown that postprandial metabolic responses are implicated as clinical signs and early markers of risk factors in a number of major diseases.

Objectives: The aim of the present study was to evaluate, during both fasting and postprandial state, glucose and plasma lipid parameters in obese patients with type 2 diabetes in order to show the main role of these parameters in health events prediction and to identify whether these responses are associated with each other. Two other groups are assessed as control; non-diabetic obese individuals and non-obese ones with type 2 diabetes.

Patients and Methods: Our study took place in two cities located in the North-Western region of Algeria (Sidi-Bel-Abbes and Mascara). During 33 months (November 2011 to July 2014), a total of 285 patients (105 males and 180 females), aged 55.41±12.77 years and distributed in three groups; diabetic overweight/obese patients (n=167), overweight/obese non-diabetic individuals (n=47) and normal weight patients with type 2 diabetes (n=71), were studied. Weight, height, waist circumference and body mass index were measured. Fasting and postprandial glucose, glycated haemoglobin (HbA1c) and lipid (total cholesterol "TC", HDL-cholesterol "HDL-c", LDL-cholesterol "LDL-c", triglycerides "TG" and apolipoproteins "apo A-I and apo B") serum profiles were evaluated. For data collection, we used a structured questionnaire to get necessary information about patient identification, general habits and diabetes and/or obesity-related information, furthermore, each patient completed a 'three-day food diary' administered at the screening. The diaries were analysed using the software program NutriSurvey.

Results: A positive effect of gender was noticed on height in the three groups of patients (p<0.001) and on weight in overweight/obese diabetic patients (p<0.001). Results for serum parameters indicated significant higher levels of glucose and HbA1c (p<0.001) in diabetic patients comparing to non diabetic ones. Assessment of lipid profiles revealed higher but non-significant levels of TC, LDL-c and TG in overweight/obese diabetic patients

compared to normal weight patients with type 2 diabetes, and this during both fasting (p=0.765, p=0.053, p=0.104) and postprandial state (p=0.266, p=0.974, p=0.064) respectively. However, a significant difference was observed for apo A-I level (p=0.027) between these two groups. On the contrary, LDL-c and TG showed higher significant levels during fasting (p=0.003, p=0.001) and postprandial state (p<0.001, p=0.001) respectively in overweight/obese non diabetic patients comparing to overweigh/obese diabetic ones. Lipid ratios comparison, during both fasting and postprandial states, indicated high significant differences of TC/HDL-c, LDL-c/HDL-c and TG/HDL-c ratios between the three groups of patients and between the two groups of overweight/obese patients. Nevertheless, no significant differences were noticed neither between the three groups (p=0.175) nor between each two groups as possible pairwise combinations when comparing apoB/apo A-I ratio. The apo B/apo A-I ratio provided best growing accurate trends when the postprandial TC/HDL-c (p<0.001, r^2=0.298, F=120.352) and LDL-c/HDL-c (p<0.001, r^2=0.234, F=86.632) ratios increase. On the other hand, dietary findings indicated a high daily total energy intake (2212.89±233.64 kcal) and lipid consumption (350.44±111.07 kcal), especially saturated ones; Myristic acid (p=0.019), Palmitic acid (p=0.001) and Stearic acid (p=0.001) in overweight/obese diabetic patients comparing to the two other groups. However, low dietary fibres intakes were recorded in all patients of both genders. Contrariwise, no distinctive deficiencies in dietary proteins and their amino acids constituents were noticed. Low dietary intake of vitamins D and B_9 and some minerals (calcium, magnesium and iodine) were recorded in all patients of both genders.

Conclusion: Management of chronic diseases such as obesity and diabetes are very important concepts, especially if these two health complications are associated with each other. Avoiding abnormalities in postprandial metabolic responses require a good mastering of body weight, nutritional parameters and pathological conditions, considering the physiological differences between males and females.

Keywords: Postprandial state, Obesity, Type 2 diabetes, Glucose, Plasma lipids.

Résumé

Contexte: L'ampleur des problèmes liés à la santé des patients atteints de diabète du type 2 est le résultat de la maladie elle-même et aussi son association avec les facteurs de risque de maladies cardio-vasculaires comme le surpoids, l'obésité et la dyslipidémie. L'objectif majeur des interventions comportementales et thérapeutiques chez les personnes diabétiques qui sont en surpoids ou obèses est basé sur les évaluations des facteurs de risque métabolique mesurés selon les recommandations en phase de jeûne. Cependant, il a été récemment montré que les réponses métaboliques postprandiales sont impliquées comme signes cliniques et marqueurs précoces de facteurs de risque dans un certain nombre de maladies graves.

Objectifs: L'objectif de la présente étude était d'évaluer, à la fois pendant la phase de jeûne et l'état postprandial, la glycémie et les paramètres lipidiques plasmatiques chez les patients obèses atteints de diabète du type 2 afin de montrer d'une part, le rôle principal de ces paramètres dans la prédiction des événements liés à la santé et pour déterminer d'autre part, si ces réponses sont associées entre eux. Deux autres groupes ont été étudiés en tant que témoins; les sujets obèses non diabétiques et les non obèses atteints de diabète du type 2.

Patients et Méthodes: Notre étude a eu lieu dans deux villes situées dans la région du Nord-Ouest d'Algérie (Sidi-Bel-Abbès et Mascara). Durant 33 mois (de novembre 2011 à juillet 2014), 285 patients (105 hommes et 180 femmes), âgés de 55.41±12.77 ans et répartis sur trois groupes; patients diabétiques du type 2 en surpoids/obèses (n=167), les sujets non diabétiques en surpoids/obèses (n=47) et les patients diabétiques du type 2 normaux pondéraux (n=71), ont été enquêtés. Le poids, la taille, le tour de taille et l'indice de masse corporelle ont été mesurés. La glycémie à jeun et postprandiale, l'hémoglobine glyquée (HbA1c) et les lipides plasmatiques (cholestérol total "CT", HDL-cholestérol "HDL-c", LDL-cholestérol "LDL-c", triglycérides "TG" et apolipoprotéines "apo AI et apo B») ont été évalués. Pour le recueil des données, nous avons utilisé un questionnaire structuré afin d'obtenir les informations nécessaires concernant l'identification des patients, leurs habitudes générales et les renseignements sur le diabète et/ou l'obésité, en outre, chaque patient a complété un «carnet alimentaire de trois jours » qui a été administré au moment de l'étude. Les carnets alimentaires ont été analysés par le biais du logiciel Nutrisurvey.

Résultats: La différence de sexe avait un effet significatif sur la taille de l'ensemble des patients au sein des trois groupes (p<0.001) et aussi sur le poids corporel des patients diabétiques en surpoids et/ou obèses (p<0.001). Les résultats des paramètres sériques ont

indiqué des niveaux significativement élevés de glucose et d'HbA1c (p<0.001) chez les patients diabétiques par rapport aux non diabétiques. L'évaluation du profil lipidique a révélé des taux élevés mais non significatifs de CT, LDL-c et TG chez les patients diabétiques en surpoids/obèses par rapport aux patients diabétiques normaux pondéraux, à la fois pendant la phase de jeûne (p=0.765, p=0.053, p=0.104) et la phase postprandiale (p=0.266, p=0.974, p=0.064), respectivement. Cependant, une différence significative a été notée concernant l'apo A-I (p=0.027) entre ces deux groupes. Les taux des LDL-c et TG ont montré des niveaux significativement élevés pendant le jeûne (p=0.003, p=0.001) et l'état postprandial (p<0.001, p=0.001), respectivement chez les patients en surpoids/obèses non diabétiques par rapport aux patients en surpoids/obèses diabétiques. La comparaison des ratios lipidiques, pendant la phase de jeûne et l'état postprandial, a révélé des différences significatives de CT/HDL-c, LDL-c/HDL-c et TG/HDL-c entre les trois groupes de patients et aussi entre les deux groupes des sujets en surpoids et/ou obèses. Néanmoins, aucune différence significative n'a été remarquée ni entre les trois groupes (p=0,175), ni entre chacun des deux groupes sur la base du rapport apo B/apo AI. Le rapport apo B/apo AI fournit des tendances croissantes plus précises lorsque les ratios CT/HDL-c (p<0,001, r^2=0.298, F=120.352) et LDL-c/HDL-c (p<0.001, r^2=0.234, F=86.632) postprandiaux augmentent. D'autre part, l'analyse des carnets alimentaires a indiqué des apports énergétiques (2212.89±233.64 kcal) et lipidiques (350.44±111.07 kcal) élevés, plus particulièrement en acides gras saturés; l'acide myristique (p=0.019), l'acide palmitique (p=0.001) et l'acide stéarique (p=0.001) chez les patients diabétiques obèses ou en surpoids par rapport aux deux autres groupes. Cependant, de faibles apports en fibres alimentaires ont été enregistrés chez tous les patients des deux sexes. En revanche, aucune déficience distinctive dans l'apport en protéines alimentaires et leurs acides aminés constituants n'a été remarquée. De faible apport alimentaire en vitamine D et B_9 et en certains minéraux (calcium, magnésium et iode) ont été enregistrés chez tous les patients, les deux sexes confondus.

Conclusion: La prise en charge des maladies chroniques comme l'obésité et le diabète est un concept très important, surtout si ces deux complications sont associées l'une à l'autre. La maîtrise des anomalies des réponses métaboliques postprandiales nécessite une bonne gestion du poids corporel, des paramètres nutritionnels et des conditions pathologiques, tout en tenant compte des différences physiologiques entre les hommes et les femmes.

Mots-clés: État postprandial, Obésité, Diabète de Type 2, Glucose, Lipides Plasmatiques.

ملخّص

خلفية: إن حجم المشكلات المتعلقة بصحة المرضى الذين يعانون من داء السكري من النوع 2 هي نتيجة للمرض نفسه وأيضا لارتباطه مع عوامل الخطر لأمراض القلب والأوعية الدموية مثل زيادة الوزن والبدانة و اعتلال نسبة الدهون في الدم. يستند الهدف الرئيسي من التدخلات السلوكية والعلاجية لدى مرضى السكري الذين يعانون من زيادة الوزن أو البدانة على تقييم عوامل الخطر الأيضية التي تقاس حسب توصيات فترة الصيام. ومع ذلك، تبين في الآونة الأخيرة أن الاستجابات الأيضية في فترة ما بعد الأكل تمثّل علامات سريرية ودلالات مبكرة لعوامل الخطر في عدد من الأمراض المستعصية.

الأهداف: الهدف من هذه الدراسة كان تقييم، أثناء فترة الصيام و مرحلة ما بعد الأكل على حد سواء،نسبة السكر و نسبة الدهون في الدم لدى المرضى الذين يعانون من زيادة الوزن أو البدانة ومرض السكري من النوع 2 ، وهذا من أجل إظهار الدور الرئيسي لهذه المعايير في توقع المضاعفات الصحية و من ناحية ثانية تحديد مدى إرتباط هذه الاستجابات مع بعضها البعض. تمت الدراسة على مجموعتين أخريين كشاهد: المرضى البدناء الغير مصابين بالسكري و المرضى الغير بدناء المصابون بمرض السكري من النوع 2.

المرضى والمنهجية : أجريت الدراسة في مدينتين تقعان في المنطقة الشمالية الغربية من الجزائر (سيدي بلعباس و معسكر) خلال 33 شهرا (من نوفمبر 2011 الى غاية يوليو 2014). شملت الدراسة 285 مريضا (105 الرجال و 180 النساء) تبلغ أعمارهم 55.41 ±12.77 عاما، موزعين على ثلاث مجموعات: مرضى السكري من النوع 2 الذين يعانون من زيادة الوزن/البدانة (ن=167)،الأشخاص ذوي الوزن الزائد/البدناء الغير مصابين بالسكري (ن=47)،و المرضى المصابين بمرض السكري من النوع 2 ذوي الوزن الطبيعي (ن=71). تم قياس الوزن و القامة و محيط الخصر و مؤشر كتلة الجسم. تم خلال فترة الصيام و مرحلة ما بعد الأكل ،قياس نسبة السكر في الدم،نسبة الهيموجلوبين السكري (HbA1c) ونسبة الدهون (الكوليسترول، نسبة البروتين الدهني المنخفض الكثافة "LDL-c"،نسبة البروتين الدهني العالي الكثافة "HDL-c"، نسبة الدهون الثلاثية و نسبة apolipoproteins "Apo A1 و Apo B"). من أجل استكمال جمع البيانات، استخدمنا استبيان للحصول على المعلومات اللازمة بشأن تحديد هوية المرضى، عاداتهم و معلومات عامة عن مرض السكري و/أو البدانة و بالإضافة إلى ذلك ، أتمّ كل مريض ملأ "يوميات غذائية لمدّة ثلاثة أيام" التي تمّ توزيعها أثناء فترة الدراسة. تم تحليل اليوميات الغذائية بأستخدام برنامج Nutrisurvey .

النتائج: كان للفرق بين الجنسين تأثير كبير على القامة لدى جميع المرضى في المجموعات الثلاث (p<0.001)، وكذلك على وزن الجسم لدى مرضى السكري الذين يعانون من زيادة الوزن و/ أو البدانة (p<0.001). أشارت نتائج تحاليل المصل لارتفاع ملحوظ في مستويات السكر و نسبة HbA1c (p<0.001) لدى مرضى السكري مقارنة مع

الغير المصابين بالسكري. تحاليل الدهون كشفت نسب مرتفعة ولكن غير ذات دلالات احصائية فيما يخّص الكوليسترول ،LDL-c و الدهون الثلاثية لدى مرضى السكري الذين يعانون من زيادة الوزن/ البدانة مقارنة مع مرضى السكري ذوي الوزن الطبيعي، و هذا سواء خلال مرحلة الصيام (p=0.104، p=0.053 و p=0.104) أو مرحلة ما بعد الأكل (p=0.266، p=0.974، p=0.064) ،على التوالي. ومع ذلك، لوحظ وجود اختلاف كبير في نسبةApo A1 (p=0.027) بين المجموعتين. بالمقابل سجلت مستويات عالية من LDL-cو الدهون الثلاثية أثناء الصيام (p=0.003، p=0.001) ومرحلة ما بعد الأكل (p<0.001، p=0.001) على التوالي، و هذا لدى المرضى البدناء الغير مصابين بالسكري مقارنة مع البدناء المصابين بالسكري. مقارنة نسب الدهون، أثناء الصيام ومرحلة بعد الأكل،كشفت عن فروق ذات دلالات إحصائية فيما يخّص الكوليسترول/HDL-c، HDL-C / LDL-cوالدهون الثلاثية/HDL-c لدى مجموعات المرضى و أيضا لدى المجموعتين من المصابين بزيادة الوزن و البدانة. مع ذلك، لا توجد فروق ذات دلالة إحصائية سواء بين المجموعات الثلاث (p=0.175) ،أو بين كل من المجموعتين على سبيل المقارنة فيما يخص Apo A1/Apo B. كما أن نسبة Apo A1/Apo Bقدمت دقة أكثر عند زيادة نسب الكوليسترول/HDL-c (p<0.001، r^2=0.298، F=120.352) HDL-c/LDL-c (p<0.001، r^2=0.234، F=86.632) أثناء فترة ما بعد الأكل. علاوة على ذلك، فإن تحليل اليوميات الغذائية أظهرت ارتفاع في كمية الطاقة المستهلكة (2212.89±233.64 كيلو حريرة) وكمية الدهون (350.44±111.07 كيلو حريرة) ،خصوصا الأحماض الدهنية المشبعة: حمض الميريستيك (p=0.019)، حمض النخليك (p=0.001) وحمض الشمعيك (p=0.001) لدى مرضى السكري الذين يعانون من البدانة أو زيادة الوزن مقارنة مع المجموعتين الأخريين. كما انه تم تسجيل انخفاض في نسبة الألياف الغذائية لدى جميع المرضى من كلا الجنسين. ومع ذلك، لم يلاحظ أي نقص واضح في البروتين والأحماض الأمينية المكونة لها. تم أيضا تسجيل انخفاض النسبة الغذائية من فيتامين د و ب 9و بعض المعادن (الكالسيوم، المغنيسيوم و اليود) لدى جميع المرضى،من كلا الجنسين.

الخلاصة : إن التحكم في الأمراض المزمنة كالبدانة ومرض السكري هو مفهوم مهم جدا، خصوصا إذا ارتبطت هذه المضاعفات مع بعضها البعض. السيطرة على الاستجابات الأيضية الغير الطبيعية في مرحلة ما بعد الأكل يتطلب تحكما جيدا في الوزن، المعلمات الغذائية و الحالات المرضية، مع الأخذ بعين الاعتبار الاختلافات الفسيولوجية بين الذكور و الاناث.

كلمات مفتاحيه: مرحلة بعد الأكل، البدانة، مرض السكري من النوع 2، السكر في الدم، الدهون.

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Chapter 5 Results

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List of Abbreviations

AA	arachidonic acid
AACE	American Association of Clinical Endocrinologists
acyl CoA	acyl co-enzyme A
ADA	American Diabetes Association
AFR	WHO African Region
AGRP	agouti-related protein
AHA/NHBLI	American Heart Association/National Heart, Blood, and Lung Institute
AI	adequate intake
ALA	α-linolenic acid
AMPK	adenosine monophosphate-activated protein kinase
AMR	WHO Region of the Americas
AP	area postrema
apo A-1	apolipoprotein A-1
apo A4	apolipoprotein A-4
apo A5	apolipoprotein A-5
apo B	apolipoprotein B
apo B_{100}	apolipoprotein B100
apo B_{48}	apolipoprotein B48
apo C2	apolipoprotein C-2
apo C3	apolipoprotein C-3
ARC	arcuate nucleus
ATP	adenosine triphosphate
BCAA	branched chain amino acids
BMI	body mass index
BP	blood pressure
C^{14}	Carbone 14
CAD	Coronary artery disease
CART	cocaine- and amphetamine-related transcript

CCK	Cholecystokinin
CDA	Canadian Diabetes Association
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
CNS	central nervous system
CV	cardiovascular
CVD	cardiovascular disease
DBP	diastolic blood pressure
DBP	Diastolic blood pressure
DCCT	Diabetes Control and Complications Trial
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DHHA	Departement for Health and Human Services
DKA	diabetic ketoacidosis
DNA	deoxyribonucleic acid
DPA	docosapentaenoic acid
DSME	diabetes self-management education
EAR	estimated average requirement
EASD	European association for the study of diabetes
EFA	essential fatty acid
EMR	WHO Eastern Mediterranean Region
ESC	European society of cardiology
EUR	WHO European Region
F HDL-c	Fasting high-density lipoprotein cholesterol
FAO	Food and Agriculture Organization
FAs	fatty acids
FDA	Food and Drug Administration
FFA	free fatty acid
FPG	fasting plasma glucose
FTO	fat mass and obesity-associated protein

G–3–P	glycerol–3–phosphate
GABA	γ–aminobutyric acid
GCKR	glucokinase regulatory protein
GI	glycaemic index
GIP	glucose–dependent insulinotropic polypeptide
GLP	glucagon–like peptide
HbA1c	glycosylated haemoglobin
HCO_3	venous bicarbonate
HDL–c	high–density lipoprotein cholesterol
HGP	hepatic glucose production
HHS	hyperosmolar hyperglycemic state
His	Histidine
HL	hepatic lipase
HMG–CoA	3–hydroxy–3–methylglutaryl–coenzyme A
HTR	Hormone replacement therapy
IAPP	islet amyloid polypeptide
IDF	International Diabetes Federation
IDL	intermediate density lipoprotein
IL–6	interleukin–6
ILE	Isoleucine
LA	Linoleic acid
LDL–c	Low–density lipoprotein cholesterol
LpL	Lipoprotein lipase
Lys	Lysine
Max.	Maximum
MCH	Melanin–concentrating hormone
Met	methionine
MetS	Metabolic syndrome
MI	Myocardial infarction
Min.	minimum

MNT	Medical Nutrition Therapy
MUFA	Monounsaturated fatty acid
NCEP/ATP	National Cholesterol Education Program/Adult Treatment Panel
NEFA	non-esterified fatty acids
NHANES	National Health and Nutrition Examination Survey
NPY	Neyropeptide Y
NRC	National research council
NTS	Nucleus of the solitary tract
OGTT	oral glucose tolerance test
OSA	Obstructive sleep apnea
OXM	Oxyntomodulin
Phe	Phenylalanine
POMC	Pro-opiomelanocortin
PPAR	peroxisome proliferator–activated receptor
PPD	postprandialy
PPG	Postprandial glucose
PPHDL-c	Postprandial high-density lipoprotein cholesterol
PPHG	Postprandial hypeglycemia
PPTG	Postprandial triglycerides
PUFA	Polyunsaturated fatty acid
PVN	Paraventricular nucleus
PYY	Peptide YY
PYY3-36	Peptide YY3-36
RDA	Recommended dietary allowance
RLP-c	Remnant-like particle-cholesterol
RNA	Ribonucleic acid
SBP	Systolic blood pressure
SCAT	Subcutaneous adipose tissue
SD	Standard deviation
SDA	Stearidonic acid

SFA	Saturated fatty acid
SNS	Sympathetic nervous system
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TC	Total cholesterol
TEI	Total energy intake
TFRNS	Task force to Revise the National Standards
TG	Triglycerides
Thr	Threonine
TNF-α	Tumour necrosis factor α
TRH	Thyrotropin-releasing hormone
TRL	Triglyceride-rich lipoproteins
Trp	Tryptophan
UCP1	Uncoupling protein 1
UKPDS	United Kingdom Prospective Diabetes Study
Val	Valine
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein
WHO	World Health Organization

Introduction

Introduction

Obesity prevalence is increasing at an alarming worldwide rate in all age-groups (Emery-Tiburcio *et al.*, 2015). Concurrently, type 2 diabetes (T2D) is continuing to be an increasing public health burden. The worldwide prevalence of diabetes among adults (aged 20-79 years) will increase from 6.4% in 2010 to 7.7% by 2033 (Shaw *et al.*, 2010). The exceeding of body weight associated with glucose intolerance and/or T2D is characterized by hyperinsulinemia, peripheral resistance to the action of insulin, hypertriglyceridemia, decreased high-density lipoprotein cholesterol and other lipid and carbohydrate changes (Weiss *et al.*, 2003; Reaven, 2005). Diabetes and obesity affect functioning, quality of life and are significantly associated with worsening of many health issues especially, those related to cardiovascular events.

Assessment of health risks, including diabetes and obesity, are measured in clinical settings and epidemiological research by referring to fasting recommendations. However, the postprandial state, a period that comprises and follows a meal, is recently receiving increased attention. The postprandial state cumulatively includes approximately half of the nycthemeral period and involves numerous finely regulated motor, secretory, hormonal and metabolic events. It was about thirty-five years ago, when Zilversmit (1979) suggested that postprandial lipemia may have a role in atherogenesis and other cardiovascular problems.

Currently, both T2D and obesity have been associated with exaggerated postprandial lipemia and glycemia. Postprandial lipid and glucose metabolism states have been investigated under some standardized circumstances, such as standardized fatty meal or an oral glucose tolerance test, which may not reflects exactly the free-living daylong situation (Ntyintyane *et al.*, 2008; Monnier *et al.*, 2011).

The regulatory pathways of postprandial metabolism are influenced by several factors including nutritional parameters modulated by dietary pattern and meal composition, life style conditions (physical activity, smoking, and alcohol consumption), physiological status (age, gender, and menopausal status), pathological conditions (diabetes, insulin resistance, and obesity) and genetics which may contribute to inter-individual variability on postprandial responses, and thereby, susceptibility to health complications (Lopez-Miranda & Marin, 2010).

The aim of the present thesis was to show, on the one hand, the main role of measuring biochemical parameters in overweight/obese patients with T2D, during both fasting and postprandial states. On the other hand, this research aimed to evaluate whether these responses are associated with each other while considering all factors influencing these metabolic responses.

The study protocol took place over three years (November 2011 to July 2014), in two cities from the north-western region of Algeria (Sidi-Bel-Abbes and Mascara). The investigation involved two other groups as control; overweight/obese individuals without diabetes and non-obese ones with T2D using a prospective multicenter case-control study.

Part One: Theoretical Considerations

CHAPTER 1

OBESITY

CHAPTER 1

OBESITY

1.1 Definition of Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. The body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in meters), is considered as a crude population measure of obesity. For a BMI of 30 kg/m^2 or more, a person is generally considered obese. For a BMI equal to or more than 25 kg/m^2, a person is considered overweight.

Since the interpretation of body fatness depends on such factors as gender, age, ethnic group, and level of physical activity, measurement of body fat is not easily done in clinical practice. Overweight is considered as a surrogate for "obesity" both clinically and epidemiologically. However, Central adiposity refers to conditions where fat is located more in the abdominal area than on the hips, thighs, or arms (Bray, 2007).

According to the World Health Organization (WHO), overweight and obesity are major risk factors for lots of chronic diseases such as diabetes, cardiovascular diseases and cancer. In a similar way, overweight and obesity are now dramatically on the rise in low, middle and in high income countries, particularly in urban settings (WHO, 2009).

1.2 Prevalence of Obesity

According to the data published on the official website of the WHO (2009), worldwide and every year, 2.8 million people die as a result of being overweight or obese,

because being overweight or obese can lead to adverse metabolic effects on blood pressure, cholesterol and triglyceride levels, and can result in increases in risks of diabetes, risks of coronary heart disease, ischaemic stroke, and a number of common cancers.

Between 1980 and 2008, the worldwide prevalence of obesity (BMI ≥30 kg/m^2) almost doubled (Figure 1.1). By 2008, 10% of men and 14% of women in the world were obese, compared with 5% of men and 8% of women in 1980. As a result, an estimated half a billion men and women over the age of 20 were estimated to be obese in 2008. In all WHO regions, women were more likely to be obese than men (WHO, 2012).

The WHO Region of the Americas are characterized by a highest prevalence of overweight and obesity (62% overweight in both sexes, and 26% obese), contrariwise the WHO South-East Asia Region are characterized by lowest prevalence (14% overweight in both sexes and 3% obese). In the other WHO Regions, over 50% of women were overweight approximately half of these overweight women were obese (WHO, 2012).

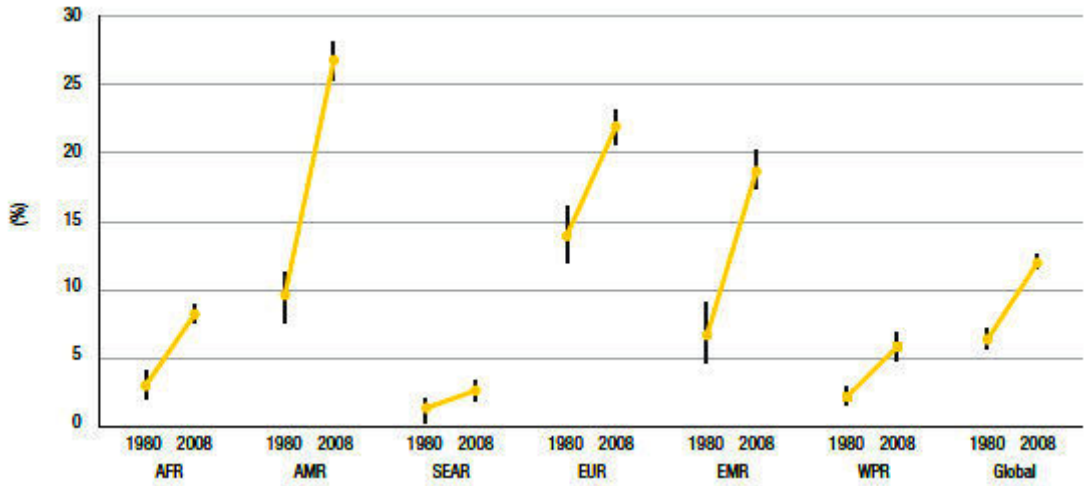


Figure 1.1 Age-standardized prevalence (%) of obesity among adults aged 20 years and over by WHO region, 1980 and 2008 (WHO, 2012)

AFR: WHO African Region, **AMR**: WHO Region of the Americas, **SEAR**: WHO South-East Asia Region, **EUR**: WHO European Region, **EMR**: WHO Eastern Mediterranean Region, **WPR**: WHO Western Pacific Region.

1.3 Aetiology of Obesity

The excess of energy consumption (dietary intake) relative to energy expenditure (energy loss via metabolic and physical activity) are the common causes of obesity, but the aetiology of obesity includes genetic, physiologic, environmental, psychological, social, economic, and even political factors that interact in varying degrees to promote the development of obesity and it is highly complex (Aronne *et al.*, 2009).

1.3.1 Nutritional causes

Overeating is highly promoted by highly caloric and fat-laden foods which are affordable and easily accessible in numerous fast food restaurants, vending machines of energy dense items in schools and offices, etc. These kinds of foods are commonly available in large portions, affecting the daily caloric intake (Rolls, 2003).

Today's and according to the fast paced life, for lots of families that are struggling to meet the economic style, the majority of products in grocery stores are non-perishable, highly processed, and pre-packaged foods. These high calorie products are frequently consumed by millions of peoples and are heavily marketed not only to adults but also to children as well (Wright & Aronne, 2012).

1.3.2 Physiological basis of obesity

Several peripheral signals and central coordination in the brain are required to help the regulation of the physiological system which can control the perturbations of the balance between food intake and energy expenditure and contribute to obesity. The hypothalamus is a central regulator in this system. This section of the brain receives information about energy balance through neuronal and hormonal signals to several tissue nuclei within it- particularly the ventro-medial, paraventricular and arcuate nuclei- and to the lateral hypothalamic area.

The arcuate nucleus has an essential role in this system; it contains two sets of neurons, one produces agouti-related protein (AGRP) and neuropeptide Y (NPY) and the other produces pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART).

The first type is orexigenic, promoting food intake and reducing energy expenditure, and the second type produces the antagonist anorexigenic effect (Barsh & Schwartz, 2002).

Peripheral endocrine signals exert their effects through this system over both long- and short-term time-frames. Insulin has a role in the central nervous system (CNS) as a signal of the level of adiposity over a moderate- to long-term period and has an anorexigenic effect through stimulation of POMC/CART neurons and inhibition of AGRP/NPY neurons (Air *et al.*, 2002).The anorexigenic hormone leptin seems to be the principal adiposity indicator

and signal of the state of nutrition, as its plasma levels are highly correlated to adipocyte number and fat content. However, leptin is involved in the long-term regulation of adiposity, and other peptides are responsible for the short-term regulation of appetite. One of these, the orexigenic peptide ghrelin, is secreted primarily by the stomach and duodenum, and shows a rise in serum levels before eating and a decrease after eating (Kohno *et al.*, 2003).

Another mediator, peptide YY3-36 (PYY3-36), is secreted from the distal gastrointestinal tract on ingestion of food, with concentrations peaking within approximately one hour. It binds to presynaptic Y2 receptors in the NPY neurons that have putative inhibitory effects, which might lead to decreased food intake.

Satiety is mediated by the response to other factors, such as gut distension and the release of the peptide cholecystokinin (CCK). The central arcuate nucleus processes these different inputs and exerts its effects by signalling to various downstream effector neurons. These include the orexigenic melanin-concentrating hormone (MCH) neurons and orexin or hypocretin neurons in the lateral hypothalamus, the thyrotrophin-releasing hormone (TRH) neurons that are involved in regulating the hypothalamic-pituitary-thyroid axis (Flier et *al.*, 2000) and the γ-aminobutyric acid (GABA)-releasing interneurons in the paraventricular nucleus (PVN), which modulate orexigenic or anorexigenic effector neurons. Further inputs to this system include the dopamine, serotonin and endocannabinoid signalling systems (Figure 1.2).

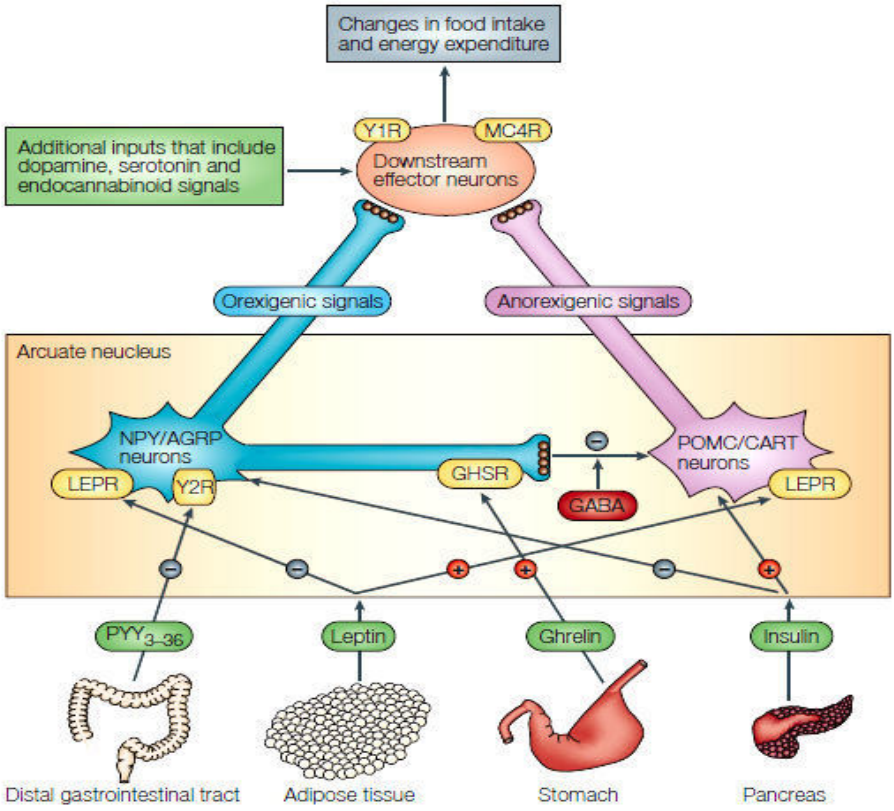


Figure 1.2 Physiological regulation of energy balance (Bell *et al.*, 2005)

This weight-regulatory system is a powerful protection against weight loss; however, the same cannot be said for weight gain. With increasing adiposity, the consequent rise in leptin has a limited effect on reducing food intake and averting obesity. The anti-obesity role of leptin is limited by cellular resistance to this signal, which might have developed in response to evolutionary pressure to promote fat storage and so protect against starvation. Various mechanisms have been proposed for the occurrence of leptin resistance, including impairment of leptin transport, as well as the presence of negative regulators of leptin and insulin signalling (Mori *et al.*, 2004).

Analysis of these physiological pathways has highlighted possible candidate genes that might underlie the genetic basis of obesity. In turn, genetic studies have contributed significantly to understanding the physiology of weight regulation, through both the use of

animal models and the investigation of the genetics of rare and common human forms of obesity (Bell *et al.*, 2005).

1.3.3 Nutrition transition

Change in diet with high fat, high energy-dense foods and a sedentary lifestyle are results of westernization, urbanisation and mechanization occurring in most countries around the world (WHO, 2000; Popkin, 2001).

Lots of factors have contributed to increased life expectancy such as the best control of infectious disease, the advancement in nutrition and hygiene. But in developed countries, infectious diseases and nutrient deficiency diseases are being replaced by other diseases such as obesity, cardiovascular diseases and diabetes (WHO, 2000).

The low price of vegetable oils and sugar means that they form, with cereals, the cheapest food ingredients around the world (Drewnowski, 2000). As a result, diets based on cereals and grain products have been reduced (Popkin, 2001) and the world average energy consumption has greatly increased, but the distribution of this consumption is not evenly throughout the world's population (Drewnowski, 2000).

Traditional diets which contain fibre and complex carbohydrates are now replaced by diets high in sugar, fat and animal products as major result of urbanisation and rising incomes (Drewnowski, 2000; Popkin, 2001). The substitution of ethnic cuisine and traditional food habits by westernised fast foods, soft drinks, increased meat consumption and the increased energy density is particularly a problem for the poor people in all countries around the world who are at risk of both obesity and micronutrient deficiencies (Pena & Bacallao, 2000; Swinburn *et al.*, 2004).

1.4 Evolution of Adipose Tissue and Obesity

1.4.1 Adipose tissue

Adipocytes are cells that secrete several proteins and bioactive peptides (adipokines) that is why adipose tissue is now considered as real endocrine organ. In addition to these functions, adipocytes are also energy depots that store triglycerides during feeding and release fatty acids during fasting (Kershaw & Flier, 2004) (Figure 1.3). Several adipose tissue-generated molecules are discovered; leptin, interleukin-6 (IL-6), and tumour necrosis factor-α (TNF- α). These molecules play an important role in energy homeostasis.

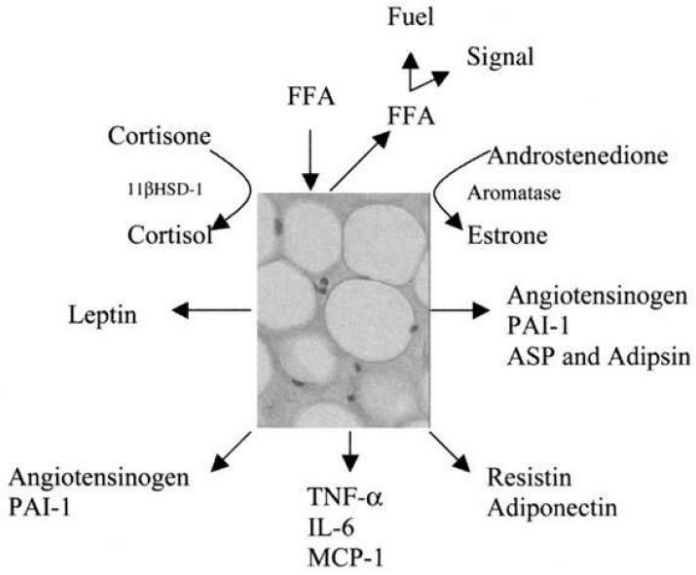


Figure 1.3 Adipocyte as an endocrine cell (Mantzoros, 2006)

1.4.2 Obesity, adipocyte hyperplasia and hypertrophy

It is well known that obesity is an unbalance between calories consumed and energy expended. The storage of excess energy in fat cells enlarged these cells in size and/or increased them in number. This hyperplasia and hypertrophy of fat cells is the pathological lesion in overweight patient (Bray, 2007).

1.4.2.1 Origin of adipocyte hyperplasia

Preclinical studies have demonstrated that adipocyte hyperplasia occurs in two steps: an increase in numbers of pre-adipocytes, followed by the differentiation of pre-adipocytes into mature adipocytes. The transition process from proliferation to differentiation in the adipocyte is tightly regulated by interaction between the cell-cycle regulators and the differentiating factors, and creates a cascade of events leading to the commitment of cells into the adipocyte phenotype (Rosen & Spiegelman 2000). This process, described as 'adipogenesis', requires a specific sequence of events to unfold, including growth arrest of proliferating pre-adipocytes, coordinated re-entry into the cell cycle with a limited clonal expansion, and growth arrest before terminal differentiation during lipid accumulation, suggesting that some cross-talk occurs between the cell cycle or the cell proliferation machinery and the factors controlling cell differentiation.

1.4.2.2 Origin of adipocyte hypertrophy

Hypertrophy is an increase in adipocyte volume. Using incorporation of environmental C^{14} as a tracer, Spalding and colleagues (2008) documented that "new adipocytes form constantly to replace lost adipocytes" and estimated the half-life of the average adipocyte to be in the order of 8.3 years. Furthermore, the same group postulated that adipocyte cell number is relatively fixed by early adulthood, and that any alterations in fat mass during adulthood are merely credited to alterations in adipocyte hypertrophy (Spalding *et al.*, 2008).

1.4.3 Obesity and adipose tissue distribution

The central change to the body in obesity is clearly the increase in the amount of adipose tissue – which may constitute more than half of total body mass in those with a BMI that is in excess of the threshold of obesity. It is not, however, only the total amount of fat that is important, but also its distribution. Thus, a more central fat deposition (‘android’ or ‘apples’, as opposed to ‘gynoid’ or ‘pears’) is associated with a greater risk of metabolic syndrome and several of the other diseases linked to obesity. A key question is why visceral fat is particularly significant in terms of obesity-associated disorders, and a long-standing position is that it is the proximity to the liver and the portal circulation that is important (Trayhurn, 2007).

1.4.3.1 Subcutaneous and visceral adipose tissue

Fat present around abdominal viscera in mesentery and momentum, known as visceral fat, is different from that present in subcutaneous areas (subcutaneous fat). The type of fat cells (Adipocytes), their endocrine function, lipolytic activity, response to insulin and other hormones differ between subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) (Mohsen Ibrahim, 2010).

Subcutaneous fat accumulation represents the normal physiological buffer for excess energy intake (high caloric diet) with limited energy expenditure (physical inactivity). It acts as a metabolic sink where excess free fatty acids (FFAs) and glycerol are stored as triglycerides (TGs) in adipocytes (Freedland, 2004). When the storage capacity of SCAT is exceeded or its ability to generate new adipocytes is impaired because of either genetic predisposition or stresses (physiological and mental stress), fat begins to accumulate in areas outside the

subcutaneous tissue, the natural store house for energy. Chronic stress leads to elevated cortesol levels that may lead to accumulation of VAT (Bjorntrop, 2001).

The anatomical and physiological differences between VAT and SCAT help explain the increased metabolic and cardiovascular risks associated with abdominal obesity. It is important to mention that the sequences proposed are being hypothetical.

1.4.3.2 Adipose tissue and insulin resistance

Adipocytes from VAT are more insulin-resistant than SCAT adipocytes. Smaller adipocytes tend to be more insulin-sensitive; large adipocytes become insulin-resistant. Amount of visceral fat is an important factor associated with variations in insulin sensitivity (Hisra & Vikram, 2003).

Insulin resistance prevents glucose and more fat from entering the cell and becoming preferentially oxidized. Subjects with visceral abdominal obesity, when compared with those with peripheral obesity, had lower glucose disposal, glucose oxidation and greater lipid oxidation.

Insulin resistance may be one of the most important factors linking abdominal visceral adiposity to cardiovascular risk (Mohsen Ibrahim, 2010).

1.4.3.3 Adipose tissue, free fatty acids lipolysis and glycerol release

Visceral adipocytes are more metabolically active and have a greater lipolytic activity than SCAT adipocytes (Lemieux & Despres, 1994). VAT is more susceptible to the catecholamine-induced lipolysis and less to the anti-lipolytic action of insulin. Free fatty acids induce insulin resistance. In the liver, insulin inhibits gluconeogenesis and glycogenlysis and stimulates glycogen formation.

The degree of FFA suppression following meal ingestion differs between abdominally and peripherally obese persons. FFAs release is greater in the abdominally obese individuals (Mohsen Ibrahim, 2010).

1.4.3.4 Adipose tissue and glucose uptake

Visceral adipose tissue has higher rate of insulin-stimulated glucose uptake compared with SCAT adipocytes (Mohsen Ibrahim, 2010).

Small adipocytes in SCAT have a high avidity for FFAs and TG uptake. The new, small, more insulin-sensitive adipocytes act as a sink or powerful 'buffers', avidly absorbing circulating FFAs and TGs in the postprandial period (Freedland, 2004).

SCAT cells may act as a buffer or sink for circulating FFAs and TGs, but once they reach their capacity they lose their protective benefit, fat begins to accumulate in tissues not suited for lipid storage (Freedland, 2004). SCAT in abdominal wall has higher uptake of TGs and larger FFA release per kilograms than femoral fat does.

1.4.4 Triglycerides synthesis

Triglycerides are synthesised from three molecules of fatty acyl (co-enzyme A) CoA that are esterified to one molecule of glycerol-3-phosphate (G-3-P), which is then derived from glucose via glycolysis, to form a molecule of triglyceride. The overall reaction sequence for triglyceride synthesis is of more than esoteric interest because the synthesis and deposition of triglyceride in adipose tissue and muscle is a major factor in regulating energy metabolism in mammals. It is generally held that insulin controls triglyceride synthesis in adipose tissue and muscle by increasing glucose uptake and regulating its conversion to G-3-P via glycolysis. Glucose metabolism via glycolysis generates dihydroxyacetone phosphate, which

can be reduced to G-3-P for triglyceride synthesis in these tissues. However, there is increasing evidence that the pathway outlined above is not the major source of G-3-P for triglyceride synthesis in mammals. In fact, a metabolic pathway termed glyceroneogenesis (i.e. the synthesis of glycerideglycerol from sources other than glycerol and glucose) is emerging as an important source of carbon for glycerideglycerol in mammals, both during starvation and after ingestion of a diet high in carbohydrates (Nye *et al.*, 2008).

1.4.5 Different origins of fatty acids

1.4.5.1 Lipolysis

The liberation of glycerol and non-esterified fatty acids is the results of white adipose tissue triacylglycerol lipolysis which are released into the vasculature and are used as energy substrates by other organs.

Lipolysis rates are regulated precisely through hormonal and biochemical signals in response to changes in nutritional state. These signals modulate the activity of lipolytic enzymes and accessory proteins, allowing for maximal responsiveness of adipose tissue to changes in energy requirements and availability (Duncan *et al.*, 2007).

Nutritional regulation of lipolysis can occur at multiple levels in response to changing metabolic conditions and nutrient intakes.

During the post-absorptive state, rapid regulation of adipose tissue lipolysis occurs in order to maintain the energy supply substrates and also to allow for efficient storage of excess fuels following a meal. In some extreme nutritional states, such as starvation or obesity, metabolic adaptations include also changes in lipolysis. And, finally, lipolysis regulation is also regulated by exposure to specific metabolically active nutrients in the diet (Duncan *et al.*, 2007).

1.4.5.2 Metabolism of brown adipose tissue

Large amounts of chemical energy as heat are dissipated by brown adipose tissue which is much evolved in mammals. Brown fat cells possess large numbers of mitochondria that contain a unique protein called uncoupling protein 1 (UCP1). UCP1 functions to dissipate the proton motive force that is normally used to drive the synthesis of cellular adenosine triphosphate (ATP) (Cannon & Nedergaard, 2004).

In small mammals, the brown adipose tissue persists as a distinct tissue, the major deposit of brown adipose tissue in newborn humans (between the shoulder blades) regresses shortly after birth. Other depots of brown adipose tissue can exist, for many decades, in adult humans but, it would have a negligible impact on whole-body energy homeostasis (Seale & Lazar, 2009).

1.5 Health Consequences of Overweight and Obesity

1.5.1 Morbidity

Above a BMI of 25 kg/m^2, morbidity for a number of health conditions increases with BMI increasing. Higher morbidity in association with overweight and obesity has been observed for hypertension, T2D, coronary heart disease (CHD), stroke, gallbladder disease, osteoarthritis, sleep apnea and respiratory problems and some types of cancer (endometrial, breast, prostate, and colon) (Must *et al.*, 1999).

Obesity is also associated with complications of pregnancy, menstrual irregularities, hirsutism, stress incontinence, and psychological disorders (depression).

The nature of obesity-related health risks is similar in all populations, although the specific level of risk associated with a given level of overweight or obesity may vary with genetic predisposition, race/ethnicity, and also with age, gender, and societal conditions. (Flegal *et al.*, 1998).

1.5.2 Early mortality

The relative increase in the risk of death associated with being obese compared to being of normal weight is not as great in older as in young adults. An assessment of 13 observational, prospective studies, in which non-hospitalised people over 65 years were followed for at least three years (Heiat *et al.*, 2001), showed an association between mortality and increased BMI in only a few, and then only above a BMI of 27-28.5, with little or no increase in mortality at any BMI for people over 75 years. Where an optimum BMI could be identified, it was usually in the range 27-30. Consistent with this, a combined analysis of the Third National Health and Nutrition Examination Survey (NHANES I–III) (1974-2000) study results found no significant increase in mortality with any degree of overweight in people over 70 years, and an increased death rate only in the 'morbidly' obese (BMI≥35 kg/m^2) among those 60-69 years (Korbints, 2008). Nevertheless, the relative risk of mortality is still increased at high BMIs until about the age of 75 years, and because of the greater background death rate in older people the absolute increase in death rate attributable to obesity is substantial; 25% of the excess deaths attributed to obesity in that NHANES analysis occurred in people over 70 years of age (Korbints, 2008).

The causes of increased mortality are essentially the same as those in younger adults: diabetes, hypertension, sleep apnoea, cardiovascular disease and an increased risk with obesity of developing certain cancers, including breast, uterus, colon and prostate (Calle *et al.*, 2003).

1.5.3 Insulin resistance

1.5.3.1 Broad definition

During insulin resistance, as reported by Wild & Byrne (2006), an impaired biological response is produces after normal or raised insulin level. Since insulin has a number of physiological actions, insulin resistance could mean impairment in acute metabolic actions, growth and development.

1.5.3.2 Specific definition in relation to metabolic syndrome

In patients with risk of T2D and metabolic syndrome, insulin resistance refers to resistance to insulin's ability to stimulate glucose uptake in insulin sensitive peripheral tissues and also its ability to suppress hepatic glucose production, promote glucose storage, inhibit ketogenesis, and suppress lipolysis (Wild & Byrne, 2006).

The abdominal accumulation of fat, in contrast to peripheral adipose tissue, is a critical determinant for insulin resistance. Fatty acids drain directly from abdominal fat into the portal system; in addition, lipolysis in central abdominal adipocytes is less suppressible by insulin (Samaras & Campbell, 2000; Lewis *et al.*, 2002). Using the hyperinsulinemic-euglycemic clamp to directly measure insulin sensitivity in vivo, Carey *et al.* (1996) showed that central abdominal fat is a stronger determinant of insulin resistance than total body, limb, or trunk fat in normal and overweight women.

1.5.4 Type 2 diabetes

Depending on age, gender, duration, distribution of adiposity, ethnicity, and compared with normal BMI of 22 kg/m^2, the risk of T2D is increased by two to eight-fold at BMI 25, 10 to 40-fold at BMI > 30, and > 40-fold at BMI > 35. Although excess fat in any

region of the body is associated with increased risk of T2D and cardiovascular disease (Wormser *et al.*, 2011), it is generally held that an accumulation of abdominal fat ('central' obesity), as indicated by an increased "waist: hip" ratio is an independent risk for T2D irrespective of the extent of obesity (Montague & O'Rahilly, 2000).

The excessive deposition of lipid in muscle and liver enhances also the risk of T2D through mechanisms of intracellular lipotoxicity which constitutes an important pathogenic link between obesity, insulin resistance and T2D. Lipotoxicity describes the detrimental cellular effects of chronically elevated concentrations of fatty acids and excess lipid accumulation in tissues other than adipose tissue.

Excess adiposity is well known to promote the onset and severity of insulin resistance, contributing to emergence and progression of T2D. Indeed, around two-thirds to three-quarters of individuals who develop diabetes have a history of significant overweight or obesity making chronic excess adiposity the strongest risk factor for T2D (Day & Bailey, 2011).

1.5.5 Endocrine and metabolic disturbances

Body metabolism is regulated through various hormones released by adipose tissue. The increase in the fat cell mass leads to imbalances in its release of hormones, which can have various metabolic effects. The metabolic complications of obesity, often referred to as the metabolic syndrome, consist of insulin resistance, often culminating in β-cell failure, impaired glucose tolerance and T2D, dyslipidemia, hypertension, and premature heart disease (Table 1.1). However, abdominal obesity, ectopic lipid accumulation, hepatic steatosis, and sleep apnea can also be included in the metabolic complications of obesity (Parati *et al.*, 2007).

In mammals, white adipose tissue is a major endocrine organ especially with identification of myriad lipid and protein signals secreted from this tissue (Havel, 2004). White adipose tissue secretes a variety of biologically active molecules, termed as adipocytokines and hormones. Of these, the hormones which play an important role in body weight regulation such as leptin, visfatin, apelin, resistin, and adiponectin (Singla *et al.*, 2010).

1.5.6 Cardiovascular diseases

The association between obesity and different forms of cardiovascular disease is due to different pathophysiological mechanisms. Obesity can cause coronary atherosclerosis through well-described and accepted mechanisms, such as dyslipidemia, hypertension, and T2D.

However, the association between obesity and cardiovascular disease could include many other factors, such as subclinical inflammation, neurohormonal activation with increased sympathetic tone, high leptin and insulin concentrations (Romero-Corral *et al.*, 2008). Furthermore, obstructive sleep apnea (OSA) and increased FFAs turnover may be due to fat deposits in specific areas of the body too with a direct role in the pathogenesis of coronary atherosclerosis, such as sub-epicardial fat as shown in Table 1.1.

Table 1.1 Metabolic and cardiovascular effects of obesity (Jimenez & S-Bergoderi, 2011)

A.	***Increased insulin resistance***
	Glucose intolerance
	Metabolic syndrome
	Type 2 diabetes
B.	***Hypertension***
	Increased plasma volume
C.	***Dyslipidemia***
	Elevated total cholesterol
	Elevated triglycerides
	Elevated LDL-c
	Elevated cholesterol other than HDL-c
	Elevated apolipoprotein B
	Elevated small dense LDL-c particles
	Reduced HDL-c
	Reduced apolipoprotein A1
	Increased free fatty acid turnover
D.	***Abnormal left ventricular morphology***
	Concentric remodelling
	Left ventricular hypertrophy
	Fat infiltration into the myocardium
E.	***Endothelial dysfunction***
F.	***Increase in systemic inflammation and prothrombotic state***
G.	***Diastolic and systolic dysfunction***
H.	***Heart failure***
I.	***Coronary Heart disease***
J.	***Atrial fibrillation***
K.	***Sudden death***
L.	***Arrhythmias and ventricular ectopias***
M.	***Obstructive sleep apnoea and sleep-related breathing disorders***

HDL-c: high-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol.

1.5.7 Cancers

In overweight individuals, many forms of cancer are significantly increased. Neoplasms of the colon, rectum, and prostate are increasing in males. However, in women, cancers of the reproductive system and gallbladder are more common (Table 1.2) (Calle *et al.*, 2003).

Table 1.2 Relation of cancer and mortality among overweight men and women (Calle *et al.*, 2003)

Men	*Women*
• **Liver**	• **Uterus**
• **Pancreas**	• **Kidney**
• **Stomach/oesophagus**	• **Cervix**
• **Colon/rectum**	• **Pancreas/ oesophagus**
• **Gallbladder**	• **Gallbladder**
• **Multiple myeloma**	• **Breast**
• **Kidney**	• **Non-Hodgkin's**
• **Non-Hodgkin's**	• **Liver**
• **Prostate**	• **Ovary**
	• **Colon/rectum**

1.5.8 Other health consequences of obesity

There is a wealth of evidence to show that excess weight is an important risk factor in the development of other illnesses, including respiratory diseases, chronic kidney diseases, musculoskeletal disorders, gastrointestinal and hepatic disorders, lower physical functioning performance and psychological problems (WHO, 2000).

1.6 Treatments of Obesity

Health improvement and risk reduction are some of wider objectives in the management and treatment of obesity. These may be achieved by modest weight loss (i.e. 5-10% of initial body weight), improved nutritional content of the diet and modest increases in physical activity and fitness (Slentz *et al.*, 2004).

1.6.1 Diet and physical activity

The use of self-recorded food diary is very important for the qualitative assessment of the diet. In addition, it helps the patient to identify perceptions and beliefs about emotional eating behaviour (cognition) and eating habits.

Many patients believe that the most effective way to lose weight and maintain a life-long ideal body weight requires a non-pharmaceutical approach (Olshansky *et al.*, 2005). This comes from the premise that energy intake must equal energy expenditure in order to have a body weight stable. Mastering eating habits and moderate-intense exercising on a regular basis would appear to be the first set of strategies to employ if an individual wants to maintain an ideal body weight or to lose weight. Although this strategy seems straight forward, many people fail to reach or maintain their optimal body weight for a variety of reasons (Wasan & Looije, 2005).

Combination of diet with exercise has a greatest impact on weight loss. For example, in their study, Foster-Schubert *et al.* (2012) reported that 60% of participants, who were on a combined diet and exercise intervention, according to the National Institutes of Health (NIH,1998) recommendations, achieved ≥10% weight loss at one year, comparing with the benefits provided from each element singly.

1.6.2 Pharmacological treatment

Pharmacological approach should be considered as part of a comprehensive strategy of disease management (Hainer *et al.*, 2008), which can help patients to maintain compliance, ameliorate obesity-related health risks and improve quality of life. It can also help to prevent the development of obesity co-morbidities (Norris *et al.*, 2005).

Drug therapy is recommended for patients with a BMI ≥ 30 kg/m^2 or a BMI ≥ 27 kg/m^2 with an obesity-related disease such as hypertension and diabetes (Hainer *et al.*, 2008). These drugs should be used according to their licensed indications and restrictions. However, an evaluation of pharmacotherapy must be performed after the first three months. Treatment can be continued if weight loss achieved is satisfactory (>5% weight loss in non-

diabetic and >3% in diabetic patients), otherwise, treatment should be discontinued in non-responders.

Few data exist to allow an evidence-based choice between the three drugs licensed and recommended for use (Orlistat, Sibutramine, Rimonabant). All three drugs produce moderate and broadly similar absolute and placebo-subtracted weight losses (Kyrou *et al.*, 2006). Currently, choice is largely determined by excluding drugs for which there are specific contra-indications (Orlistat; for chronic mal- absorption syndrome and cholestasis), (Sibutramine; for psychiatric illness, concomitant use of monoamine oxidase inhibitors) and Rimonabant; for history of treatment of major depressive illness and/or ongoing antidepressive treatment.

1.6.3 Surgical procedures

The safety and efficacy of surgery has shown a remarkable improvement. If costs of drugs, supplements, complications and side effects are taken into account, calculations of cost per kilogram of maintained weight loss have shown a "break even". For ethical and scientific reasons, randomisation studies of surgery and non-surgical treatment cannot be done. Furthermore, it is very difficult to retain participants in non-surgical treatment long enough to provide meaningful comparable outcome data (Kral, 2006).

A BMI of ≈40 or 35-40 with obesity related co-morbidity is widely accepted indication for surgery since the 1960s. Recommended requirements for surgery include that patients should have seriously tried to lose weight by other means and usually a minimum of 20-25 years and a maximum of 60-65 for age criteria (Kral, 2006).

CHAPTER 2

TYPE 2 DIABETES

CHAPTER 2

TYPE 2 DIABETES

2.1 Definition of Type 2 Diabetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Based on aetiology, diabetes comprises type 1 diabetes (T1D), T2D, other specific types, and gestational diabetes. T2D is the most common with approximately 90-95% of those with diabetes (WHO 2006; ADA, 2011).

Increased morbidity and mortality are now substantially related to diabetes; long-term complications of diabetes include increased risk of macrovascular complications such as ischemic heart disease, stroke and peripheral vascular disease, and microvascular damage such as retinopathy, nephropathy, and neuropathy.

Guideline recommendations were developed using the WHO definition of diabetes, which requires high plasma glucose levels sufficient to put the individual at risk of the microvascular complications of diabetes. This definition was re-confirmed by WHO in 2006, but, like earlier versions, it does not contain a specific definition for T2D. A person is regarded as T2D patient if he or she does not have T1D (rapid onset, often in childhood, insulin-dependent, ketoacidosis if neglected), monogenetic diabetes or other medical conditions or treatment suggestive of secondary diabetes.

2.2 Diagnosis of Diabetes

Glycosylated haemoglobin (HbA1c) ≥ 6.5% and/or fasting plasma glucose (FPG) ≥ 126 mg/dL (7.0 mmol/L), are considered as first diagnosis methods of T2D. Fasting is defined as no caloric intake for at least eight hours. Two hours plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT) is also used. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L); in the absence of unequivocal hyperglycemia, the result should be confirmed by repeat testing (ADA, 2014).

2.3 Epidemiology of Type 2 Diabetes

2.3.1 Aetiology and pathophysiology

Type 2 diabetes is typically a multifactorial disease caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance, environmental factors, obesity, overeating, lack of exercise and stress, as well as aging (Figure 2.1).

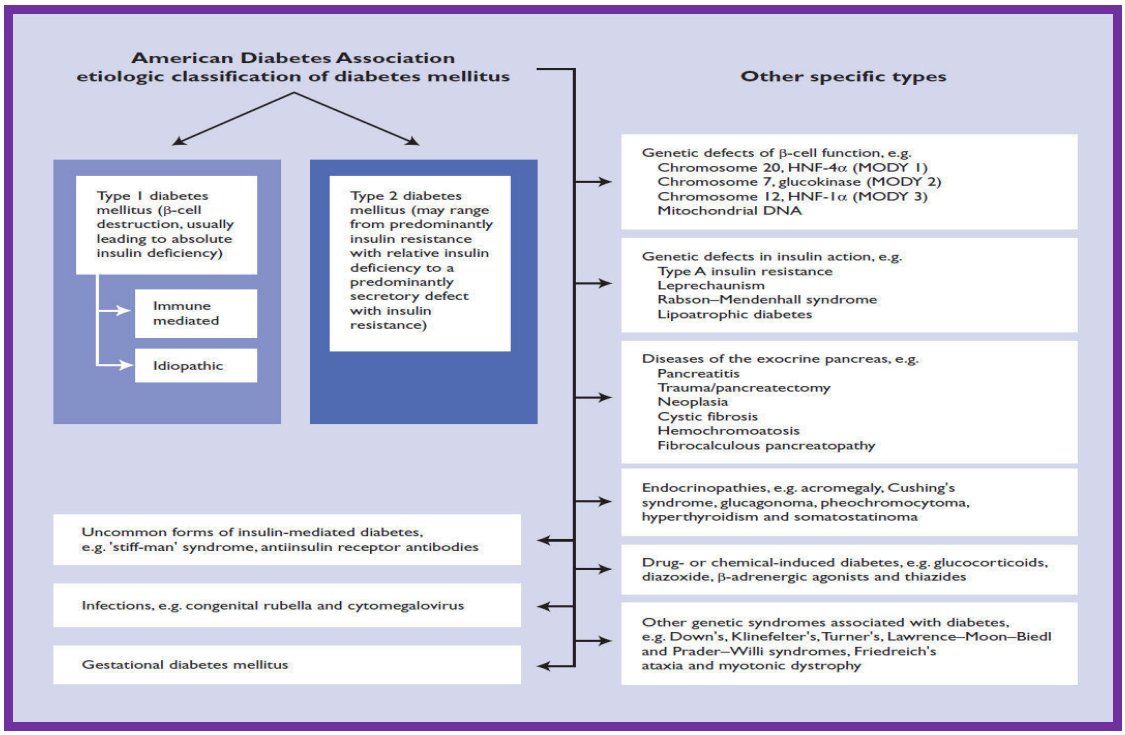


Figure 2.1 Etiologic classification of diabetes according to the American Diabetes Association (ADA) (ADA, 2007)

The nomenclature has changed from insulin-dependent diabetes to T1D and from noninsulin diabetes mellitus to T2D. All forms of diabetes are characterized according to their known aetiologies, immunologic, genetic or otherwise (Scobie, 2007).

2.3.2 Genetic factors

According to Poulsen *et al.* (1999), the high concordance in monozygotic twins (over 80%) and the 50 % decline in dizygotic twins provide compelling evidence for a genetic component in the aetiology of T2D. Data from various studies showed the role of a genetic basis for measures of both insulin sensitivity and insulin secretion (Elbein *et al.*, 1999).

The observation that the disease prevalence varies substantially among ethnic groups that share a similar environment supports also the idea that genetic factors contribute to predisposition to the disease (Diamond, 2003). Other source of evidence for genetic

contribution is the familial aggregation, if we admit that families also share common environmental traits.

2.3.3 Environmental factors

Aging, obesity, insufficient energy consumption, alcohol drinking, smoking, etc. are independent risk factors of pathogenesis of T2D. Obesity due to a lack of exercise is accompanied by a decrease in muscle mass, induces insulin resistance, and is closely associated with the rapid increase in the number of middle- and high-aged patients.

Obesity and deterioration of glucose tolerance are generally due to changes in dietary energy sources, such as the increase in fat intake and consumption of simple sugars, the decrease in starch and dietary fiber intake. Even overweight (BMI of 25kg/m^2) causes a four (4) to five (5) fold increase in the risk of developing diabetes, if accompanied by the increase in visceral fat mass (Kaku, 2010).

2.3.4 Abnormalities in insulin secretion

Several factors may lead to abnormalities in insulin secretion. The loss of the first phase insulin response to an intravenous glucose load is common in T2D, although this abnormality may be acquired secondary to glucotoxicity. However, many other abnormalities have been shown to disturb the delicate balance between islet neogenesis and apoptosis.

In patients with early stages of T2D, insulin resistance can be compensated by an increase in insulin secretion leading to normal glucose tolerance (Ramlo-Halsted & Edelman, 1999).

With increasing insulin resistance, the fasting plasma glucose will rise, accompanied by an increase in fasting plasma insulin levels, until a fasting plasma glucose level is reached when the β-cell is unable to maintain its elevated rate of insulin secretion at which point the fasting plasma insulin declines sharply. Hepatic glucose production will begin to rise. When fasting plasma glucose reaches high levels, the plasma insulin response to a glucose challenge is markedly blunted (Leahy, 2005). Although fasting insulin levels remain elevated, postprandial insulin and C-peptide secretory rates are decreased. This natural history of T2D starting from normal glucose tolerance followed by insulin resistance, compensatory hyperinsulinemia and then by progression to impaired glucose tolerance and overt diabetes has been documented in a variety of populations (Scobie, 2007).

Some acquired defects in T2D may lead to impairment of insulin secretion. Clinical studies in both man and animal have supported the concept of glucotoxicity, whereby an elevation in plasma glucose levels, in the presence of a reduced β-cell mass, can lead to a major impairment in insulin secretion. Lipotoxicity has also been implicated as an acquired cause of impaired β-cell function (Prentki & Nolan, 2006). Patients with T2D exhibit a reduced response of the incretin glucagon-like peptide (GLP)-1 in response to oral glucose, while GLP-1 administration enhances the postprandial insulin secretory response and may restore near-normal glycaemia.

Amyloid deposits "islet amyloid polypeptide (IAPP)" or amylin in the pancreas are frequently observed in patients with T2D and have been implicated as a cause of progressive β-cell failure. However, definitive evidence that amylin contributes to β-cell dysfunction in humans is lacking (Scobie, 2007).

2.3.5 Insulin resistance in type 2 diabetes

In both lean and obese individuals with T2D, insulin resistance is the main feature. Since hyperinsulinemia is a potent inhibitor of hepatic glucose production (HGP) and an excessive rate of HGP is the major abnormality responsible for the elevated fasting plasma glucose in T2D, it follows that there must be a hepatic resistance to the action of insulin. The liver is also resistant to the inhibitory effect of hyperglycaemia on hepatic glucose output. Most of the increase in HGP can be accounted for by an increase in hepatic gluconeogenesis (Edgerton *et al.*, 2001).

Muscle is the major site of insulin-stimulated glucose disposal in humans. Muscle represents the primary site of insulin resistance in T2D subjects leading to a marked blunting of glucose uptake into peripheral muscle. In contrast, splanchnic tissues, like the brain, are relatively insensitive to insulin with respect to stimulation of glucose uptake. Following glucose ingestion both impaired suppression of hepatic glucose production and decreased muscle glucose uptake are responsible for the observed glucose intolerance leading to hyperglycaemia (Abdul-Ghani *et al.*, 2006).

There is a dynamic relationship between insulin resistance and impaired insulin secretion. Insulin resistance is an early and characteristic feature of T2D in high-risk populations. Overt diabetes develops only when the β-cells are unable to increase sufficiently their insulin output to compensate for the defect in insulin action (insulin resistance).

Insulin resistance in T2D is primarily due to post-binding defects in insulin action. Diminished insulin binding is modest and secondary to down-regulation of the insulin receptor by chronic hyperglycaemia. Post-binding defects that have been recognized include reduced insulin receptor tyrosine kinase activity, insulin signal transduction abnormalities,

decreased glucose transport, diminished glucose phosphorylation and impaired glycogen synthesis activity. Quantitatively, impaired glycogen synthesis represents the major abnormality responsible for insulin resistance in T2D diabetic patients (Scobie, 2007).

2.3.6 Obesity

The adipocyte is not a simply storage depot for fat, it is a real endocrine organ which is gaining appreciation since it is capable of secreting a number of adipose-tissue-specific or enriched hormones, known as adipocytokines or adipokines. Indeed, besides non-esterified fatty acids (NEFA), adipocytes secrete various cytokines, among which leptin, TNF-α, resistin and adiponectin (Greenberg & McDaniel, 2002; Ravussin & Smith, 2002).

Despite the fact that TNF-α and resistin clearly inhibit cellular insulin action, the role of leptin in carbohydrate metabolism remains unclear (Greenberg & McDaniel, 2002). However, most studies were performed in rodents and the contribution of these two cytokines to hyperglycaemia of T2D is still controversial in humans.

Among the various adipocytokines, adiponectin appears to play an important role in carbohydrate and lipid metabolism (Chandran *et al.*, 2003). Adiponectin, which is synthesized solely in adipose tissue, appears to be a major modulator of insulin action. In contrast to other adipocytokines, adiponectin is characterized by lower (and not higher) circulating levels in obese patients. In addition, whereas leptin is more positively related to subcutaneous than to intra-abdominal fat, adiponectin is more strongly negatively related to intra-abdominal than to subcutaneous fat. The levels of leptin are reduced in T2D and a strong positive relationship between insulin sensitivity and adiponectin levels has been described in various populations. Thus, low adiponectin levels could contribute to peripheral insulin resistance in T2D. The mechanism of adiponectin synthesis needs to be elucidated, as

do the signals that reduce adiponectin expression in adipocytes with increasing adiposity. Similarly, the molecular mechanisms by which adiponectin exerts its multiple functions still remain unresolved as well as its potential role in the development of T2D in obese subjects, especially those with intra-abdominal adiposity (Chandran *et al.*, 2003).

2.3.7 Evaluation parameters of glycemic control

The usual metabolic control means of diabetes based on fasting glucose, postprandial glucose and glucosuria are not sufficient. Although and since the advent of new techniques: HbA1c and fructosamine, monitoring of the diabetic patient has become more objective.

2.4 Complications of Type 2 Diabetes

2.4.1 Acute complications of type 2 diabetes

2.4.1.1 Hypoglycemia

Alarming results have been provided by many surveys about the prevalence of hypoglycaemia. The Diabetes Control and Complications Trial (DCCT, 1997) reported a threefold increase in severe hypoglycaemia and coma in patients treated intensively comparing to those treated conventionally. According to the United Kingdom Prospective Diabetes Study (UKPDS, 1998), hypoglycemia is relatively common in T2D, with prevalence rates of 70-80 % in clinical trials using insulin to achieve good metabolic control.

2.4.1.2 Diabetic ketoacidosis

In patients with T2D, diabetic ketoacidosis (DKA) is a serious and potentially life-threatening complication (Kearney & Dang, 2007) which represents a state of insulin deficiency with a concurrent elevation in counterregulatory hormones (Magee & Bhatt, 2001). DKA is the result of carbohydrate, protein and lipid metabolism dysregulation. Insulin

deficiency along with an increase in counterregulatory hormones (glucagon, cortisol, catecholamine, and growth hormone) lead to the development of hyperglycaemia, ketosis, and acidosis (Kitabchi *et al.*, 2006).

The symptoms of the DKA are generally nausea, vomiting, thirst, polyuria and, occasionally, abdominal pain accompanied by signs of dehydration, acidotic respiration, ketones on the breath, hypothermia and altered consciousness (Scobie, 2007).

2.4.1.3 Hyperosmolar non-ketoacidotic coma

Middle-aged patients and elderly people who have undiagnosed T2D are generally affected by hyperosmolar non-ketoacidotic coma. Many factors can contribute to this coma such as; infection, diuretic therapy and ingestion of glucose-rich drinks. As for the treatment of diabetic ketoacidosis, insulin replacement is recommended for the treatment of the hyperosmolar non-ketoacidotic coma (Scobie, 2007).

2.4.2 Chronic complications of type 2 diabetes

2.4.2.1 Micro vascular complications

Generally, the injurious effects of hyperglycemia are separated into microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke). For physicians, it is important to understand the relationship between diabetes and vascular disease because the prevalence of diabetes continues to increase, and the clinical armamentarium for primary and secondary prevention of these complications is expanding too (Fowler, 2008).

Diabetes is a group of chronic diseases characterized by hyperglycaemia. Modern medical care uses a vast array of lifestyle and pharmaceutical interventions for preventing and

controlling hyperglycaemia. In addition to ensuring the adequate delivery of glucose to the tissues of the body, treatment of diabetes attempts to decrease the likelihood that the tissues of the body are harmed by hyperglycaemia (Özkaya, 2012).

a. Diabetic retinopathy

Diabetic retinopathy is considered as the most common microvascular complication of diabetes. In the United States alone, retinopathy is responsible of ~ 10,000 new cases of blindness every year (Fong *et al.*, 2004).

The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycaemia. The development of diabetic retinopathy in patients with T2D was found to be related to both severity of hyperglycaemia and presence of hypertension in the U.K (UKPDS, 1998).

Initially, retinopathy begins like small haemorrhages in the middle layers of the retina, which clinically appear as "dots" and therefore are frequently referred to as "dot haemorrhages." Hard exudates are caused by lipid deposition that typically occurs at the margins of haemorrhages. Micro-aneurysms are small vascular dilatations that occur in the retina, often as the first sign of retinopathy. They clinically appear as red dots during retinal examination.

Retinal oedema results from microvascular leakage and is indicative of compromise of the blood-retinal barrier. The appearance is one of grayish retinal areas. In some situations, retinal oedema may require intervention because it is usually associated with visual deterioration (Watkins, 2003).

Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous haemorrhage. White areas on the retina ("cotton wool spots") can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous haemorrhage and traction retinal detachment. With no intervention, visual loss may occur. Laser photocoagulation can often prevent proliferative retinopathy from progressing to blindness; therefore, close surveillance for the existence or progression of retinopathy in patients with diabetes is crucial (Watkins, 2003).

b. Diabetic nephropathy

Diabetic nephropathy represents one of leading cause of renal failure. In diabetic patients, nephropathy is defined by proteinuria > 500 mg in 24 hours, but this is preceded by lower degrees of proteinuria called also "microalbuminuria". Microalbuminuria is defined as albumin excretion of 30–299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both T1D and T2D.

At the time they are diagnosed with diabetes, more than 7% of patients with T2D may already have microalbuminuria (Gross *et al.*, 2005).

In the kidney, many pathological changes can occur including increased glomerular basement membrane thickness, micro-aneurysm formation, mesangial nodule formation, and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy (Fowler, 2008).

c. Diabetic neuropathy

According to the American Diabetes Association (ADA), diabetic neuropathy is defined as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" (ADA, 2007). The risk of developing diabetic neuropathy, as with other microvascular complications, is proportional to both the magnitude and duration of hyperglycemia, whereas some individuals have a genetic predisposition for developing such complications.

2.4.2.2 Macro vascular complications

Despite microvascular disease is a major concern in diabetic patients, most patients with long-term T1D and most patients with T2D will die because of cardiovascular disease. An excess mortality owing to coronary artery disease in diabetic patients, particularly females, was observed comparing to the non-diabetic control population. As well as there is an increased mortality too, due to peripheral vascular disease (Scobie, 2007).

Reduction in the cardiovascular burden requires a multifactorial approach, encompassing control of glycaemia, reduction in traditional cardiovascular risk factors (hypertension, hyperlipidaemia), use of antiplatelet agents and angiotensin-converting enzyme inhibitors, and aggressive treatment of coexisting cardiovascular disease.

The process of atherosclerosis is the central pathological mechanism in macrovascular disease, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system.

In T2D, in addition to atheroma formation, there is strong evidence of increased platelet adhesion and hypercoagulability. Impaired nitric oxide generation and increased free radical formation in platelets, as well as altered calcium regulation, may promote platelet aggregation. Elevated levels of plasminogen activator/inhibitor type 1 may impair fibrinolysis too in patients with diabetes. The combination of increased coagulability and impaired fibrinolysis likely further increases the risk of vascular occlusion and cardiovascular events in T2D (Beckman *et al.*, 2002).

Diabetes increases the risk that an individual will develop cardiovascular disease (CVD) including atherosclerosis, myocardial infarction (MI), stroke, and peripheral vascular disease. It is two to four times higher in adults with diabetes to die from heart disease and stroke than in adults without diabetes (CDC, 2005). Furthermore, heart disease and stroke account for approximately 65% of deaths in people with diabetes.

T2D typically occurs in the setting of the metabolic syndrome, which is associated with abdominal obesity, hypertension, hyperlipidemia, and increased coagulability. Table 2.1 lists specific definitions of the metabolic syndrome from major medical organizations (Alberti *et al.*, 2006).

Table 2.1 Definitions of metabolic syndrome from major organizations

Organization	WHO	NCEP ATP III	AHA/NHLBI	AACE	IDF
Diagnostic criteria	Insulin resistance (refer to glucose intolerance section) + any 2 components	Three or more components	Any 3 of the 5 components	Diagnosis based on clinical judgment of risk factors	Central obesity + any 2 components
Abdominal obesity	BMI>30 kg/m^2 and/or waist: hip ratio Men>0.9 Women>0.85	Waist circumference Men>40 Inches (102 cm) Women>35 inches (88 cm)	Waist circumference Men≥40 inches Women≥35 inches	BMI>25 kg/m^2 or waist circumference Men >40 inches Women>35 inches	Waist circumference Europids Male≥94 cm Female≥80 cm South Asians and Chinese Male≥90 cm Female≥80 cm Japanese Male≥85 cm Female≥90 cm Specific data not yet available for ethnic groups
Dyslipidemia Elevated TGs Reduced HDL-c	TGs>150 mg/dl HDL-c Men>35 mg/dl Women <39 mg/dl	TGs≥150 mg/dl HDL-c Men<40 mg/dl Women<50 mg/dl	TGs≥150 mg/dl or drug therapy for elevated TGs HDL-c Men<40 mg/dl Women<50 mg/dl or drug therapy for low HDL-c	TGs>150mmHg HDL-c Men<40 mmHg Women<50 mmHg	TGs≥150 mg/dl or specific treatment for elevated TGs HDL-c Men<40 mg/dl Women<50 mg/dl or specific treatment for low HDL-c
Blood pressure	SBP≥140 mmHg or DBP≥90 mmHg or drug therapy for hypertension	BP≥130/85 mmHg	SBP≥130 mmHg or DBP≥85 mmHg or drug therapy for hypertension	BP>130/85 mmHg	SBP≥130mmHg or DBP≥85 mmHg or treatment of previously diagnosed hypertension
Glucose intolerance	Any of the following T2D impaired FPG impaired glucose tolerance If normal FPG (<110 mg/dl), glucose update bellow the lowest quartile	FPG≥110 mg/dl	FPG≥100 mg/dl or drug therapy for elevated glucose	FPG 110-126 mg/dl 2-hour postglucose challenge 140-200 mg/dl	FPG≥100mg/dl or previously diagnosed T2D if FPG>100 mg/dl, oral glucose tolerance test strongly recommended but not required for diagnosis of syndrome

AACE : American Association of Clinical Endocrinologists ((Einhorn *et al.*, 2003); **AHA**/**NHBLI**: American Heart Association/National Heart, Blood, and Lung Institute (Grundy *et al.*, 2004); **BMI**: body mass index; **BP**: blood pressure; **CVD**: cardiovascular disease; **DBP**: diastolic blood pressure; **FPG**: fasting plasma glucose; **HDL–c**: high-density lipoprotein cholesterol; **IDF**: International Diabetes Federation (Alberti *et al.*, 2006); **NCEP ATP**: National Cholesterol Education Program Adult Treatment Panel (NCEP, 2002); **SBP**: systolic blood pressure; **T2D**: type 2 diabetes mellitus; **TGs** = triglycerides; **WHO**: World Health Organization (Alberti *et al.*, 1998; WHO, 1999).

2.4.3 Hypertension

In Western societies, diabetes and hypertension are both common conditions. When compared with the non-diabetic population, it has often been stated that hypertension is at least twice as common in diabetic subjects, although the prevalence is clearly dependent on the definition of hypertension itself.

Many authors have shown that hypertension progression is an independent predictor of T2D (Movahed *et al.*, 2010). Several possible factors are likely causes of the association between T2D and hypertension:

- Markers of endothelial dysfunction are associated with new-onset of diabetes (Meigs *et al.*, 2006), and endothelial dysfunction is closely related to blood pressure and hypertension;
- Markers of inflammation such as C-reactive protein have been consistently related to incident of T2D (Hu *et al.*, 2004), and to increasing blood pressure levels, suggesting that inflammation might be another explanatory factor for the association between blood pressure, the metabolic syndrome, and incident T2D (Ridker *et al.*, 2003);
- Insulin resistance could be another potential link between blood pressure levels and the incidence of T2D;
- There is a strong correlation between blood pressure and BMI and risk of T2D (Czernichow *et al.*, 2002).

2.4.4 Metabolic complications of diabetes

2.4.4.1 Diabetic dyslipidemia

Diabetes in adults is associated with a high risk of vascular disease as shown on Figure 2.2 Aggressive management of all cardio vascular (CV) risk factors, including dyslipidemia, is therefore generally necessary (Gæde *et al.*, 2003). In T2D patients, the most common lipid pattern consists of hypertriglyceridemia (hyper-TG), low high-density lipoprotein cholesterol (HDL-c) and normal plasma concentrations of low-density lipoprotein cholesterol (LDL-c). Nevertheless, with the presence of even mild hyper-TG, LDL-c particles are typically small and dense and are more susceptible to oxidation. Chronic

hyperglycemia promotes the glycation of LDL-c and both these processes are believed to increase the atherogenicity of LDL-c. Plasma lipid and lipoprotein concentrations may be normal, in patients with T1D, but there may be oxidation and glycation of the lipoproteins, which may impair their function and/or enhance their atherogenicity.

The vast majority of patients with established T2D should be considered at high short-term risk (DCCT/EDIC, 2005). In the NHANES III, the highest prevalence of CHD (19.2%) was observed in people aged more than 50 years with both diabetes and the metabolic syndrome, compared to people with neither condition. The prevalence of the metabolic syndrome was extremely high (86%) among people with diabetes (Alexander *et al.*, 2003).

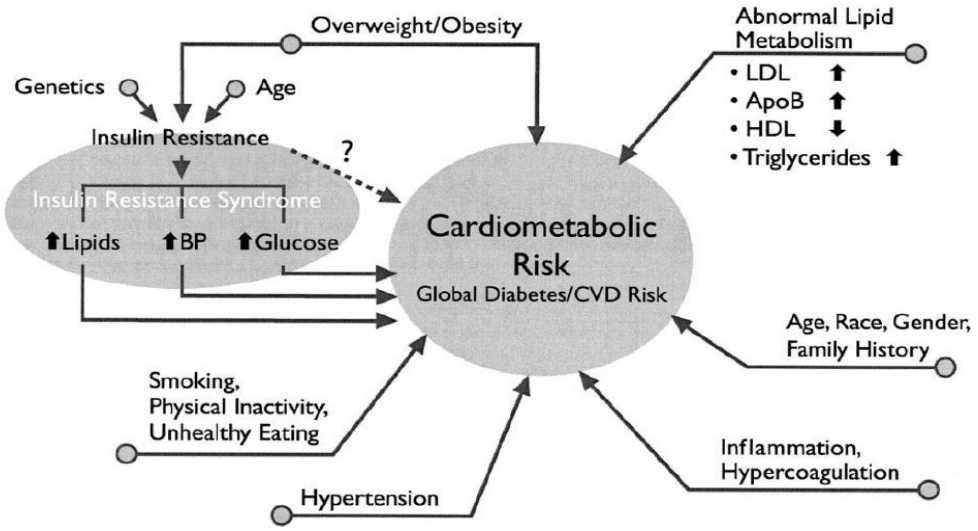


Figure 2.2 Factors contributing to cardiometabolic risk (Brunzell *et al.*, 2008)

a. LDL cholesterol

Generally, the most common LDL-c level in diabetes is "borderline high" (130-159 mg/dl), however, there is no difference of LDL-c levels in people with diabetes compared to people without diabetes who are matched for age, sex, and body weight. LDL-c does not

play less of a role in cardiovascular risk in people with T2D. In fact, LDL-c levels may underestimate cardiovascular risk in diabetes (Buse *et al.*, 2007). A large number of small, dense particles characterize the LDL-c fraction in diabetic individuals. These particles, which are exceptionally atherogenic, contain less cholesterol than normal-sized LDL particles (Marcovina & Packard, 2006). Thus, levels of LDL may appear deceptively "normal" in cholesterol measurements.

b. Total cholesterol (TC)/HDL-c ratio

For CV risk, the TC to HDL-c (TC/HDL-c) ratio is a highly sensitive and specific index. This simple lipid ratio is recommended as a secondary goal of therapy. An elevated TC/HDL-c ratio in the face of an optimal LDL-c of ≤2.0 mmol/L is usually associated with a low HDL-c and/or elevated TG. This form of dyslipidemia is more amenable to lifestyle modification (increase in physical activity and weight reduction) and improvement in glycemic control than is an isolated LDL-c elevation (CDA, 2006).

c. Apolipoprotein (apo) B, apo B/apo A-1 ratio

There is one particle of apo B per LDL, very low-density LDL and intermediate-density lipoprotein particle. Comparing to LDL-c, Apo B particles have repeatedly been shown to be a better risk marker for CVD events; that is why the measurement of apo B and its monitoring in response to lipid-lowering therapy has been advocated by some (Barter *et al.*, 2006).

In patient with hyper-TG, the measurement of apo B is clinically more useful, because this measurement provides an indication of the total number of atherogenic lipoprotein particles in the circulation. In such cases, with the knowledge of the apo B level

we can have a good idea about the aggressiveness with which lipid-lowering therapy is pursued (Walldius *et al.*, 2001).

In the circulation, apo A-1 is a surrogate marker of the number of HDL particles (there may be 2 to 4 apo A-1 molecules per HDL particle). The apo B/apo A-1 ratio was found to be the best predictor of CVD risk (Yusuf *et al.*, 2004).

Despite the fact that both apo B and the apo B/apo A-1 ratio have been shown to predict CVD events, it does not exist a real clinical trial evidence for specific targets for these indices in individuals with or without diabetes.

2.4.4.2 Hypoglycaemia

Previously, hypoglycaemia was relatively rare in T2D comparing to T1D, but in the past few years, hypoglycaemia is becoming an increasing consequence of T2D treatments. Furthermore, hypoglycaemia in T2D is associated with longer length of hospital stay, greater cost, and higher mortality during hospitalization (Turchin *et al.*, 2009).

There are two major categories of hypoglycaemia symptoms; Neurogenic signs associated with elevated epinephrine levels include shakiness, anxiety, nervousness, palpitations, sweating, dry mouth, pallor, and pupil dilation (Cryer, 2004) and the cholinergic mediated symptoms include diaphoresis, hunger, and paresthesias.

2.4.4.3 Lactic acidosis

Severe lactic acidosis is defined as a high anion gap metabolic acidosis with a blood lactate concentration >5.0 mmol/L (normal 0.4-1.2 mmol/L). The pathological elevation of lactate and hydrogen ions may result from overproduction or delayed clearance of lactate, or

a combination of both (English & Williams, 2004). This is a severe accident in diabetes mellitus, a single intensive care is likely to prevent a fatal outcome.

2.4.5 Mortality due to diabetes

Among leading causes of death for diabetic patients, cardiovascular disease can account for 50% or more of deaths due to diabetes in some populations. Since many countries don't have any data on diabetes-related mortality, the estimation of the number of deaths due to diabetes is becoming a big challenge. To provide a more realistic estimate of mortality, a modelling approach is used in the International Diabetes Federation (IDF) Diabetes Atlas to estimate the number of deaths that can be attributed to diabetes in 2011(Figure 2.3) (IDF, 2012).

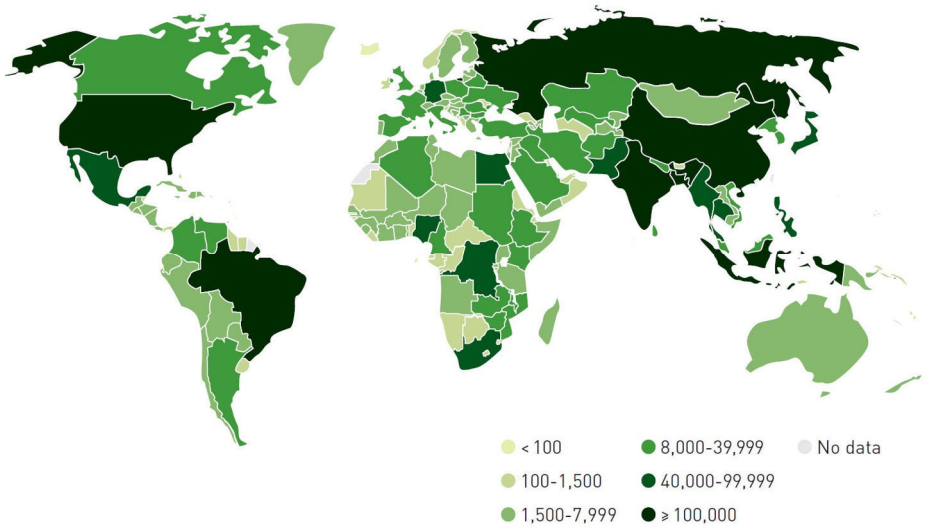


Figure 2.3 Deaths attributable to diabetes (20 to79 years), 2011(IDF, 2012)

During 2011, more than 4.6 million people aged 20-79 years died from diabetes, accounting for 8.2% of global all-cause mortality of people in this age group. This estimated number of deaths is similar in magnitude to the combined deaths from several infectious diseases that are major public health priorities, and is equivalent to one death every seven

seconds. Forty-eight percent of deaths due to diabetes are in people under the age of 60. The highest number of deaths due to diabetes is in countries with the largest numbers of people with diabetes; India, China, United States of America, and the Russian Federation (IDF, 2012).

2.5 Treatment Goals for Patients with Type 2 Diabetes

Prevention of macrovascular complications such as hypertension, stroke, and heart disease is the main goal of effective diabetes management (Table 2.2), as well as debilitating acute and chronic microvascular complications, including nephropathy, neuropathy, and retinopathy. Other complications of diabetes which include birth defects and spontaneous abortion, immune system dysfunction, and periodontal disease should also be managed (ACE, 2002).

Table 2.2 Treatment goals for adults with type 2 diabetes (LaRocque *et al.*, 2009)

Hemoglobin A1c level	Glucose level (mg/dL)	Blood pressure level (mmHg)	Cholesterol-Lipid profile (mg/dL)
<7.0%	• Preprandial plasma Glucose range: 70-130 • Peak postprandial Plasma glucose<180	• Systolic <130 • Diastolic<80	• LDL-c <100 • LDL-c<70 with high CVD risk • Triglycerides <150 • HDL-c Men>40 Women>50

LDL-c: low-density lipoprotein; HDL-c: high-density lipoprotein; CVD: cardiovascular disease.

2.5.1 Food intake and life style conditions

Medical Nutrition Therapy (MNT), which is an important component of healthy lifestyle, is considered as a cornerstone of diabetes prevention and management. MNT has been shown to accrue sustained reduction in HbA1c in diabetic patients (Funnell *et al.*,

2010). However, in non-diabetic individuals, this medical nutrition therapy can improve the lipid profile and blood pressure (Van Horn *et al.*, 2008). In nowadays, the subject of interest is the optimum dietary macronutrient composition; however, dietary measures are effective in weight reduction irrespective of the composition as it had been shown in several studies, provided there is adequate energy restriction, reduction in saturated fat to less than 7%, and adequate provision of dietary fibre (Stern *et al.*, 2004).

In overweight and obese patients, a reduction of as few as 4.5 to 9 kg or 5% to 10% of an individual's body weight can improve hyperglycaemia and insulin action, decrease fasting blood glucose concentrations, and reduce the need for some medications in certain individuals with T2D.

In patients with diabetes, glycemic control can be improved by a lower consumption of total and saturated fat and processed foods, and higher consumption of dietary fibres, whole grains, fruits, and vegetables. In clinical trials, nut consumption increases satiety, have a neutral effect on glucose and insulin, and a beneficial effect on lipid profile (Brennan *et al.*, 2010). The use of artificial sweeteners according to Food and Drug Administration (FDA) recommendations can be safe, otherwise, they may cause diarrhea. Although diabetic subjects may have increased oxidative stress, placebo-controlled trials have not demonstrated any clear benefit attributable to antioxidant supplementation (Song *et al.*, 2009).

2.5.2 Exercise

One of the most important risk factors for T2D is the sedentary lifestyle. Benefits of physical activity in the prevention and management of T2D have been shown in several studies on prediabetic and diabetic subjects (Knowler *et al.*, 2002).

Exercise may reduce the accumulation of fatty acids in the myocytes by oxidizing them. Therefore life style modification, by focusing on the weight loss and increase in the physical activity, may prevent progression toward frank diabetes in people who have an impaired glucose tolerance test (Bonen *et al.*, 2006).

According to 14 trials that investigated the effect of exercise on glycemic control in diabetic patients, an engaging in a structured moderate exercise program for about 50 minutes three times a week during eight weeks has shown a reduction of 0.7% in HbA1c level (Boulé *et al.*, 2001). In patients with diabetes, the mechanisms by which exercise produces positive results, include improvement in body fitness, insulin sensitivity, glucose disposal in the skeletal muscle and expression of nitric oxide synthase in the endothelial cells. However, an adjustment in the dosage of medications and monitoring of blood glucose during exercise remains an important measure.

2.5.3 Drugs treatments

2.5.3.1 Oral antidiabetes agents

Several classes of oral antidiabetes medications are available to help lower blood glucose levels (Figure 2.4). However, the oral antidiabetic therapy is not really effective in individuals who have had T2D for more than 10 years and in those who use insulin as therapy with more than 20 units per day, or in individuals who are extremely thin.

An algorithm is outlining by the ADA in addition to lifestyle modifications, this algorithm describe the initiation of well-validated core oral hypoglycaemic medications for diabetes control. In this algorithm, metformin is "Step 1" in medication based on effectiveness, cost, and reversal of metabolic changes in diabetes. Oral sulfonylurea is added as Step 2 therapy, however, Step 3 progresses to intensive insulin therapy. Less-validated

alternatives include the use of a thiazolidinedione, GLP-1 agonist, or basal insulin between Step 1 metformin and the addition of intensive insulin therapy in Step 3 (Nathan *et al.*, 2009).

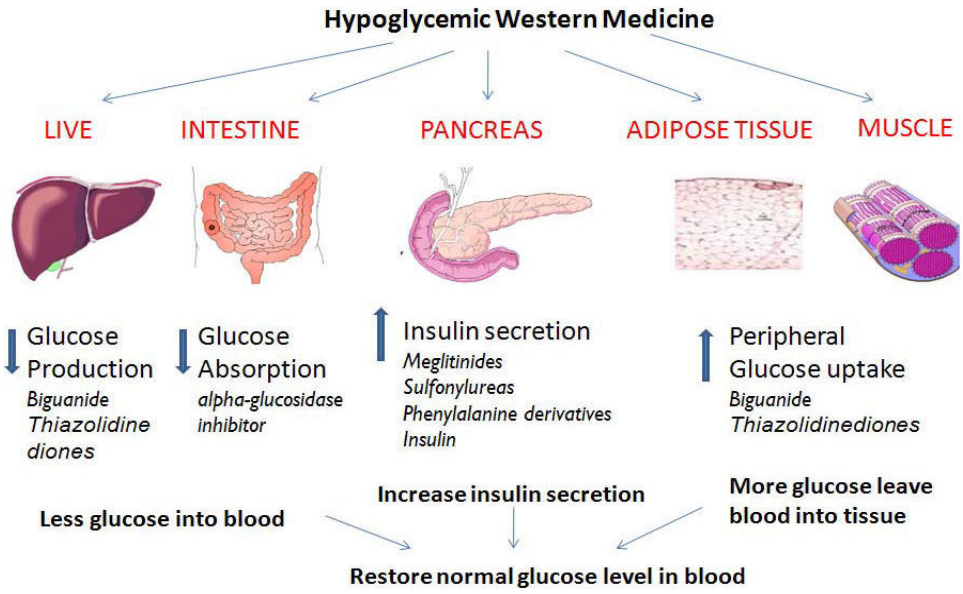


Figure 2.4 Sites of western medicine in diabetes treatment (Hui *et al.*, 2009)

a. Metformin

The use of the biguanide metformin as treatment for T2D dates back to 1957 in Europe and Canada, but in the United Stated it was licensed in 1995 because fear of lactic acidosis, a rare but fatal complication that was associated with phenformin, the first biguanide to be introduced. In patients treated with metformin, lactic acidosis is estimated to occur in 1 case per 100 000 patients, especially in the setting of renal failure, with creatinine clearance of less than 30 ml/min (Salpeter *et al.*, 2006).

For the treatment of T2D, metformin is established as a first-line antidiabetic therapy. Metformin is considered as an "insulin sensitizer" because its use lowers glucose levels through nonpancreatic mechanisms without increasing insulin secretion. That is why the use

of metformin is considered as one of the few antidiabetic therapies that do not cause hypoglycaemia (Rojas & Gomes, 2013).

The mechanism of metformin action involves suppression of endogenous glucose production by the liver which is mainly due to a reduction in the rate of gluconeogenesis and a small effect on glycogenolysis. In addition there is an increase in peripheral glucose disposal that arises largely through increased non-oxidative glucose disposal into skeletal muscle. Another beneficial effect of metformin which can be observed in adipose tissue is the reduction in fatty acids. Moreover, metformin activates the adenosine monophosphate-activated protein kinase enzyme (AMPK) resulting in the inhibition of key enzymes involved in gluconeogenesis and glycogen synthesis in the liver while stimulating insulin signalling and glucose transport in muscles (Lim *et al.*, 2010) (Figure 2.5). Long term treatment with metformin reduces cardiovascular mortality in overweight T2D patients.

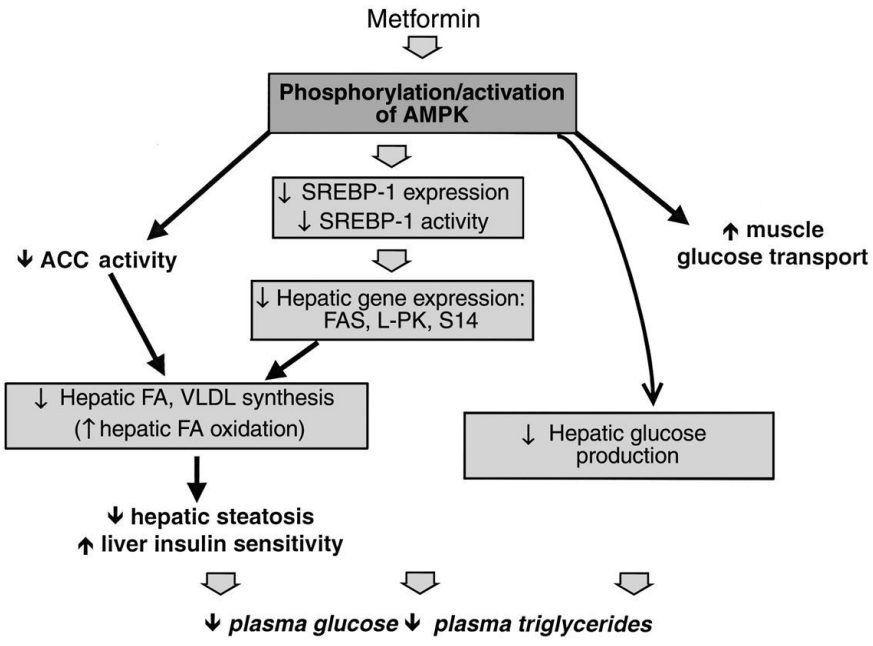


Figure 2.5 Model for the mechanism by which metformin mediates effects on lipid and glucose metabolism (Zhou *et al.*, 2001)

b. Sulphonylurea

In adults with T2D, when metformin alone is inadequately effective, it is recommended that a sulfonylurea be added to metformin (CADTH, 2010).

Sulphonylureas are remarkably safe and free from side effects, although rare toxic effects have been reported, including rashes and jaundice and these in the seven available sulphonylureas. Only one sulphonylurea should be used at a time since there is nothing to be gained from any combination of these drugs and there is no evidence that any one drug is likely to be more successful than another (Watkins, 2003).

Selecting of sulphonylurea is based on the shorter acting; however, metabolised drugs such as gliclazide or glipizide are suitable for all ages and for those with renal impairment as well. Glibenclamide, which has the advantage of once-daily use, is still suitable for younger patients, but is contraindicated in the elderly. Glimepiride which may cause less hypoglycaemia is also given once daily. Hazardous hypoglycaemia is generally caused by excessive doses, and it is thus usual to start treatment with the smallest useful dose. Sulphonylurea should be stopped or at the very least the dose substantially reduced if hypoglycaemia occurs in a patient taking this drug. The Chlorpropamide is now obsolete, it has a considerable long half-life, thus increasing the risk of hypoglycaemia (Watkins, 2003).

c. Thiazolidinediones

Thiazolidinediones, widely prescribed drugs for T2D patients, are known to increase insulin sensitivity via activation of peroxisome proliferator-activated receptor (PPAR)-receptors.

However, if studies in animal showed that the use of thiazolidinedione as treatment was associated with bone loss in a mouse model, it is not known whether the use of this drug has any effect on bone mass and thereby increases risk of fracture in T2D patients (Rzonca *et al.*, 2004). Even though, a recent study has suggested that thiazolidinedione use may cause bone loss in older women with T2D.

d. Glucagon–like peptide 1 analogues

Given by injection, GLP-1 analogues are a new class of glucose lowering drugs that mimic the action of an endogenous gastrointestinal hormone GLP-1, it is an incretin hormone that is released into the circulation in response to food. Through the stimulation of glucose dependent insulin and biosynthesis, GLP-1 analogues regulate glucose levels in the body. GLP-1 analogues suppress glucagon secretion, delaying gastric emptying and promoting satiety as well (Baggio *et al.*, 2004).

The action of the GLP-1 analogues is glucose-dependent, when the plasma glucose level increases, the effect of GLP-1 agonists on insulin secretion increase also, this results in a greatest effect in hyperglycaemic conditions, and little or no effect when the blood glucose concentration is less than 3.6 mmol/l (65 mg/dl). This should reduce the occurrence of hypoglycaemia. The GLP-1 analogues have been reported to produce weight loss too in patients with T2D (Norris *et al.*, 2009).

e. Lipas inhibitors (Orlistat)

Orlistat is an anti-obesity drug with a well documented efficacy in weight reduction and maintenance (Filippatos *et al.*, 2009). Orlistat has beneficial effects on metabolic indices, reducing the incidence of T2D in patients with impaired glucose tolerance (Torgerson *et al.*, 2004). It was also shown to decrease LDL-c levels to a greater degree than expected from

weight loss alone. In obese patients with hypercholesterolaemia, Orlistat – Fluvastatin, Orlistat – Simvastatin and Orlistat – Cerivastatin combinations led to pronounced weight loss and a greater decrease in LDL-c concentration compared with statin monotherapy (Derosa *et al.*, 2003).

2.5.3.2 Insulin

In about 30% of patients with T2D, glycemic control based on lifestyle modifications and/or antidiabetic agents may fail and therefore, will eventually require insulin therapy. A specific schedule of insulin therapy for all individuals does not exist; factors to consider include an individual's weight, level of exercise, diet, alcohol use, insulin resistance/β-cell failure, and daily pattern of fasting/post-prandial hyperglycemia.

Indications for giving insulin to T2D patients who are inadequately controlled despite adherence to their recommended diet and oral hypoglycaemic agents are as follows:

- Rapid and sustained weight loss with persistent symptoms. Insulin treatment in these patients almost always results in a substantial improvement in health (Watkins, 2003);
- Treatment with insulin can give a good improvement in about 50% of non-obese patient without symptoms whose weight is stable and who is conscientious with existing medication;
- An obese patient whose weight is stable presents an even more difficult issue. Ensuring that they are taking their medication together with intensification of diet is the correct management, but sometimes insulin may be needed in order to reduce long-term complications during the following decade or more. However, a reduction of HbA1c of approximately 2% together with

weight gain of around 5-7 kg can be expected. Unfortunately improvement in glycemic control is not always achieved. Some patients prefer not to take insulin after all explanations which have been presented. Reluctant patients can be given a three-month trial of insulin and then make their decision, which experience shows to be usually affirmative (Watkins, 2003) ;

- In some patients with intercurrent illness, insulin therapy is often required. Many disorders, notably infections, increase insulin resistance, leading to the temporary need for insulin. Withdrawal of insulin after recovering from the illness is important provided adequate control is achieved and maintained.

Corticosteroids always exacerbate hyperglycaemia and often precipitate the need for insulin. This should not deter doctors from prescribing them when they are needed (Watkins, 2003).

2.5.4 Therapeutic education

According to Task Force to Revise the National Standards (TFRNS, 1995), the process of teaching people to manage their diabetes, called Diabetes Self-Management Education (DSME), which has been considered as an important part of the clinical management of diabetes since the 1930s (Figure 2.6). The ADA recommends assessing self-management skills and knowledge of diabetes at least annually and providing or encouraging continuing education (ADA, 2001).

Within the first few weeks of diagnosis of the diabetic disease, DSME should be introduced. A minimum of 10 to 12 hours of education should be provided to all patients within twelve months of the initial diagnosis. The preferred method is that instruction be provided within 12 weeks of the initial diagnosis.

Available teaching and learning strategies should be explored and considered for motivating learners. Options include:

- Brief lecture
- Discussion; more participatory and active
- Demonstration for teaching psychomotor or social skills
- Printed materials for reinforcement
- Audio visual aides to enhance presentation
- Role playing
- Games
- Computers to increase patient knowledge and increase problem-solving skills
- Patient examples

The aim of most diabetes educators is to have patients who are motivated, responsible and committed. Education is much more rewarding for participants and educators if participants are active and committed learners (Anderson, 2001).

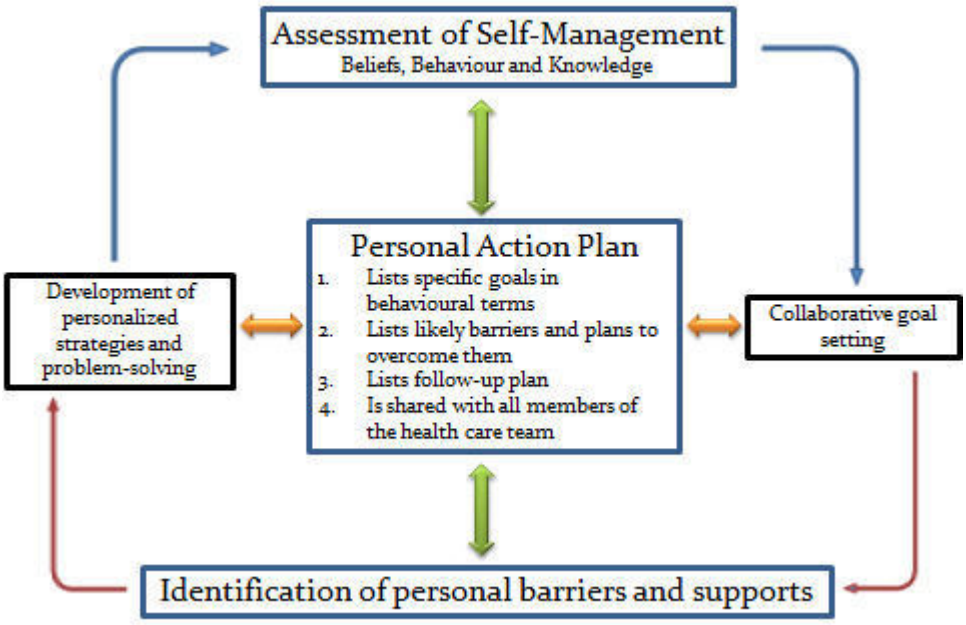


Figure 2.6 Behavioural principles expressed as self-management action steps (Glasgow & Bull, 2001)

CHAPTER 3

METABOLISM OF POSTPRANDIAL STATES

CHAPTER 3

METABOLISM OF POSTPRANDIAL STATES

3.1 General Information about Food Intake Control

3.1.1 Food intake

For a long, food intake was considered as a matter of will. It was also assumed that obese people could lose weight only by making an effort to control their food intake. This concept has been widely reviewed during the last century with the gradual discovery of many hormones involved in the control of food intake and energy expenditure. Indeed, the concept of secretin is introduced by showing that injection of rat intestinal mucosa stimulated pancreatic secretions. These discoveries have accelerated with the development of new technologies for biochemistry (Carrel & Giusti, 2009).

Endocrine regulation of food intake uses feedback mechanisms. The brain can modify food intake and energy expenditure according to hormonal signals received which give the exact energetic state of the body. These interactions create a real axis adipotrope comparable to other endocrine axes. This axis is composed of adipotrope messengers promoting food intake or decreasing the food intake. Moreover, these signals are often classified as long-acting elements, such as leptin which inform the agency of the state of the stocks of energy, or short-term, such as gastrointestinal hormones which inform satiety. Finally, food intake is vital for the survival of the organism, the system is composed of elements effects often redundant (Carrel & Giusti, 2009).

3.1.2 Different periods after food intake

3.1.2.1 Postprandial state

With respect to glucose, the postprandial state is the period of 4h following the ingestion of a meal (Figure 3.1). Dietary carbohydrates are progressively hydrolyzed through several sequential enzymatic actions during this postprandial period. The glucose postprandial excursions can rapidly return to baseline levels within 2h through the insulin action, the overall period of absorption has approximately a 4h duration that corresponds to the postprandial state (Monnier & Colette, 2009).

Unlike circulating carbohydrates that normally show only transient elevations (as glucose) following a meal, circulating triglycerides show pronounced elevation (postprandial lipemia) within an hour of meal ingestion and can remain elevated for 5-8h following consumption of a typical fat containing meal (30-60 g fat) (Lairon *et al.*, 2007).

3.1.2.2 Postabsorptive state

This state takes place during the 6-h period that follows the postprandial period. In nondiabetic individuals, glucose concentrations remain within a normal range during this postabsorptive state through the breakdown of the glycogen (glycogenolysis) stored during the postprandial period.

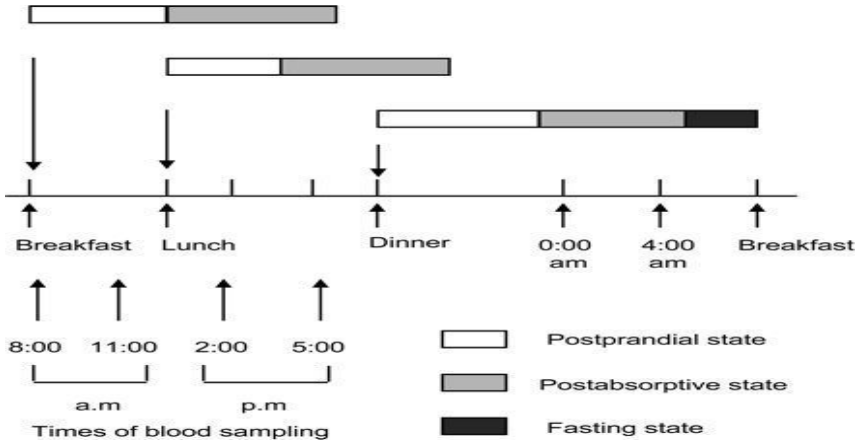


Figure 3.1 Duration of the postprandial, post-absorptive, and fasting states (Monnier & Colette, 2009)

The postprandial and the post-absorptive states last for 4 and 6 h, respectively. Therefore, the cumulative duration of postprandial state is ~12 h, which is equivalent to a full half-day period of time, and the "real" fasting state is limited to a 3-h time interval at the end of the night (Monnier & Colette, 2009).

3.1.2.3 Fasting state

At the end of the post-absorptive state begins the fasting state (10–12 h after the beginning of the last meal intake). During the fasting state, plasma glucose is maintained at a near normal level by the gluconeogenesis: glucose derived from lactate, alanine, and glycerol.

In non diabetic individual who respect a daily intake of three meals in fixed hours, the 24-h period of the day can be divided into three periods corresponding to fasting, postprandial, and post-absorptive states. The real fasting period is only limited to a 3- to 4-h period of time at the end of the night (Monnier & Colette, 2009).

3.1.3 Regulation of food intake

3.1.3.1 Central nervous system factors

The hypothalamus is the part of central nervous system which contributes to regulate the food intake. The hypothalamus is linked to specific parts of the brain that are known to modify feeding behavior, specifically the paraventricular nuclei and the nigrostriatal tract. These areas of the brain respond to various neurotransmitters as well as sympathetic nervous system activity (Figure 3.2). In general, when the sympathetic nervous system activity increases, the food intake will decrease, and vice versa (Gibney *et al.*, 2009).

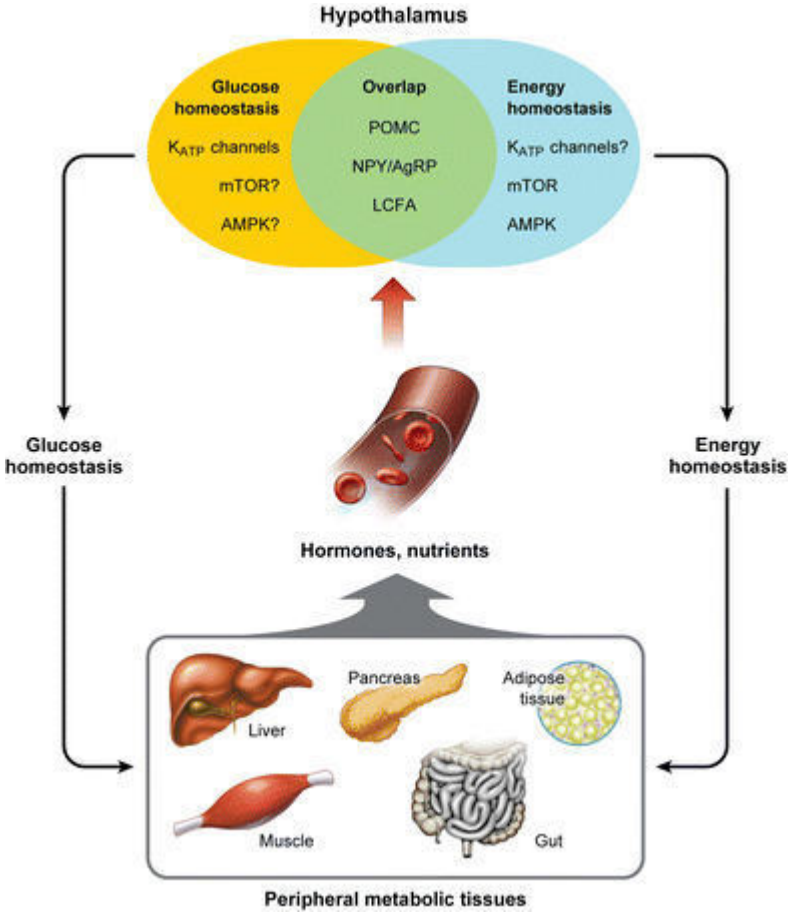


Figure 3.2 Central and peripheral regulation of food intake (Sandoval *et al.*, 2008)

3.1.3.2 Circulating factors

In the digestive system, food is broken down into its basic components (i.e., carbohydrates are broken down to glucose molecules, protein to amino acids, and fats or triglycerides to glycerol and fatty acids), the circulating levels of some of these breakdown products increase in the blood simultaneously. These basic components are further metabolized, primarily in the liver, or used for immediate energy (e.g., in muscle or brain). There is evidence to suggest that this resultant metabolism, especially in the liver, may in turn regulate food intake. After meal consumption, the circulating levels of nutrients fall (within minutes for glucose, several hours for triglycerides) and the feelings of hunger return. The link from nutrient metabolism to central control of food intake occurs through signals from the liver to the brain via the vagus nerve (Figure 3.3). Thus, circulating factors provide a link between the digestive system and the central nervous system, which provides another system for regulating food intake (Gibney *et al.*, 2009).

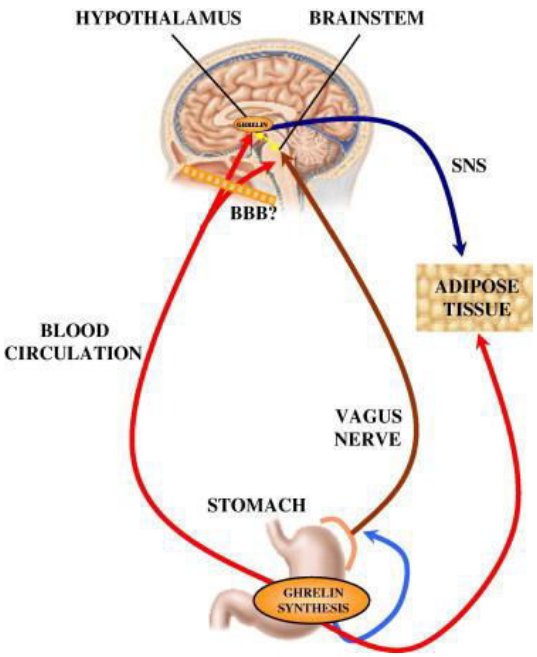


Figure 3.3 Ghrelin synthesis and actions (Castañeda *et al.*, 2010)

The circulating ghrelin is predominantly synthesized and secreted by the stomach and small intestine. Ghrelin has an effect on food intake and body weight through the activation of NPY and AGRP neurons in the hypothalamus. Ghrelin-induced orexigenic effects are abolished by vagotomy. Ghrelin also promotes adiposity through the melanocortin-sympathetic nervous sytem (SNS) pathway.

3.1.3.3 Signals from the periphery

Leptin is a hormone that is produced by fat cells and communicates with the central nervous system through leptin receptors in the hypothalamus. Rare forms of obesity in humans are due to a reduced in leptin production, or lack of sensitivity of the hypothalamus to leptin which lead to troubles in food intake. Hormones with a central effect on appetite, and amongst them leptin, are classified into two categories: (1) the adiposity signals, which provides information on body fat stores to the CNS and (2) the satiety signals which are released in response to food intake are involved in short-term regulation of energy intake. Insulin, leptin, and adiponectin, are known as adiposity signals and are considered as long-acting signals reducing energy intake. Ghrelin is a hormone of satiety signals, which is secreted in the stomach, in addition to others short-acting gut- and pancreas-derived satiety signals cholecystokinin (CCK), peptide YY (PYY), GLP-1, oxyntomodulin (OXM), and pancreatic polypeptide. The arcuate nucleus (ARC) of the hypothalamus plays an important role in appetite regulation through the existence of receptors of peripheral satiety signals. The ARC contains NPY- and AGRP-expressing neurons acting to stimulate food intake along with the adjacent POMC and cocaine- and amphetamine-regulated transcript (CART)-expressing neurons which inhibit feeding. Besides the ARC, the nucleus of the solitary tract (NTS) and the area postrema (AP), which are connected to the hypothalamic nuclei controlling food intake, receive appetite-regulating inputs from vagal afferents and circulating factors (Gibney *et al.*, 2009).

3.1.3.4 External factors

Many externals and nonphysiological factors can modify food intake. Depression, which is considered as psychological factor, may lead to either increased or decreased food intake, or changes in the consumption of specific types of foods.

Some of the specific properties of food can make it more or less appealing, even when food is available; our food intake is thereby modified by food properties. Important physical characteristics of food include taste, texture, colour, temperature, and presentation. Other cultural factors play a central role in influencing food intake such as time of day, social factors, peer influence, and cultural preferences (Gibney *et al.*, 2009).

3.2 Metabolism of Postprandial Glycemia

3.2.1 Physiologie of postprandial glycemia

3.2.1.1 Definition of postprandial glucose

Many factors are used for determining the profile of postprandial hyperglycemia including the timing, quantity, and composition of the meal, the carbohydrate content of the meal, and the resulting secretion of insulin and inhibition of glucagon secretion. The optimal time to measure postprandial blood glucose levels is an open question because in individuals with and without diabetes the absorption of food continues until 5 to 6 hours after a meal (Table 3.1).

Table 3.1 Summary of Post-prandial Glucose Guidelines

Organization, Year	**HbA1c (%)**	**FPG (mg/dl)**	**PPG (mg/dl)**	**PPD Timing**
IDF, 2007	<7	<110	<140	1-2 hours postprandialy
ESC/EASD, 2007 Type 1 diabetes Type 2 diabetes	 ≤6.5 ≤6.5	 ≤108 ≤108	135-160	'Peak'
ADA, 2007	≤6.5	70-130	<180	1-2 hours postprandialy
AACE, 2007	≤6.5	110	<140	2 hours postprandialy

IDF: International Diabetes Federation (IDF, 2007); ESC/EASD: European Society of Cardiology/European Association for the Study of Diabetes (Rydén *et al.*, 2007); ADA: American Diabetes Association (ADA, 2007); AACE: American Association of Clinical Endocrinologists (AACE, 2007).

According to the WHO definition, the post-prandial blood glucose levels are generally <120mg/dl in healthy non-diabetic subjects and rarely exceed 140mg/dl (WHO, 2006). However, according to the IDF, post-prandial hyperglycemia is defined as a plasma glucose level exceeding 140mg/dl (IDF, 2007).

The postprandial hyperglycemia develops in coincidence with an impairment or absence of the first-phase insulin response, a decrease in insulin sensitivity in the peripheral tissues, and decreased suppression of hepatic glucose output after meals due to insulin deficiency (Pratley & Weyer, 2001). In T2D patients, the post-prandial hyperglycemia is one of the earliest abnormalities of glucose homeostasis, and worsens in progressing with fasting hyperglycemia (Monnier *et al.*, 2007). The contribution of PPG is generally highest at HbA1c levels of ≈6.5%, when FPG levels are close to normal values. This contribution become lowest at HbA1c levels >8%, when the FPG level predominates. Thus, diabetes

management based on the triad; PPG, HbA1c and FPG could optimize glycemic control (Ceriello & Colagiuri, 2008).

3.2.1.2 Factors influencing postprandial hyperglycemia

a. Quantity and quality of ingested carbohydrates

Postprandial glycemia can be affected by both quantity and quality of ingested carbohydrates.

Despite the fact that the source of carbohydrates and nature can have a great influence on postprandial glycemia, the current dietary recommendations focus more on quantity rather than quality of carbohydrates. Research on glycemic index (GI) indicates that even when foods contain the same amount of carbohydrate, there are up to fivefold difference in glycemic impact (Foster-Powell *et al.*, 2002).

The ADA acknowledges that postprandial glucose may be reduced by the use of low GI foods, despite that there is insufficient evidence of long term benefit to recommend their use as a primary strategy in diabetic treatment (ADA, 2002). In contrast, the European Association for the Study of Diabetes recommends the substitution of low-GI foods for high-GI foods (EASD, 2000).

b. Gastric emptying

Several studies suggest that the rate of gastric emptying plays a crucial role in postprandial glycemia in diabetic patients (Horowitz *et al.*, 2002). Many therapies are currently in development designed to improve postprandial glycemic control by modulating gastric emptying through the rate of nutrient delivery into the small intestine (Horowitz *et al.*, 2002).

Postprandial hyperglycemia can be the result of an accelerated emptying by a mismatch between absorbable food in the duodenum and endogenous insulin release, which leads to transiently elevated blood glucose concentrations. However, slowing gastric emptying pharmacologically in diabetic patients might also be a good means to improve control of postprandial blood glucose (Phillips *et al.*, 1993).

In diabetic patients suffering from gastroparesis, the control of PPG by further delaying gastric emptying can possibly worsen their gastroparetic symptoms (Gonlachanvit *et al.*, 2003).

c. Intraluminal digestion of carbohydrates and intestinal absorption of glucose

In the diet, carbohydrates constitute the most important energy-containing components. The energetic value of most carbohydrates is 17.5 kj per g, so that a daily diet of 400 g carbohydrates covers 7 000 kj, which is 56% of the usable energy in a diet of 12 500 kj daily.

Digestion of starches to simple hexoses occurs in two phases: In the mouth, the starch is under the action of salivary amylase (ptyalin) which constitutes the luminal phase, but most of this phase occurs in the upper small intestine as pancreatic α-amylase reach the chime (Figure 3.4). The starch polymer is reduced to maltose, maltriose and α-limit dextran or dextrins. The brush-border phase begins when the three substrates are pushed through the intestine. Some of the substrate molecules get into contact with the brush-borders of the absorbing mucosal cell via the unstirred water layer. Enterocytes carry disaccharidases and trisaccharidases (oligosaccharidases) on their surface that cleave these substrates to glucose-G (Paulev & Zubieta-Calleja, 2012).

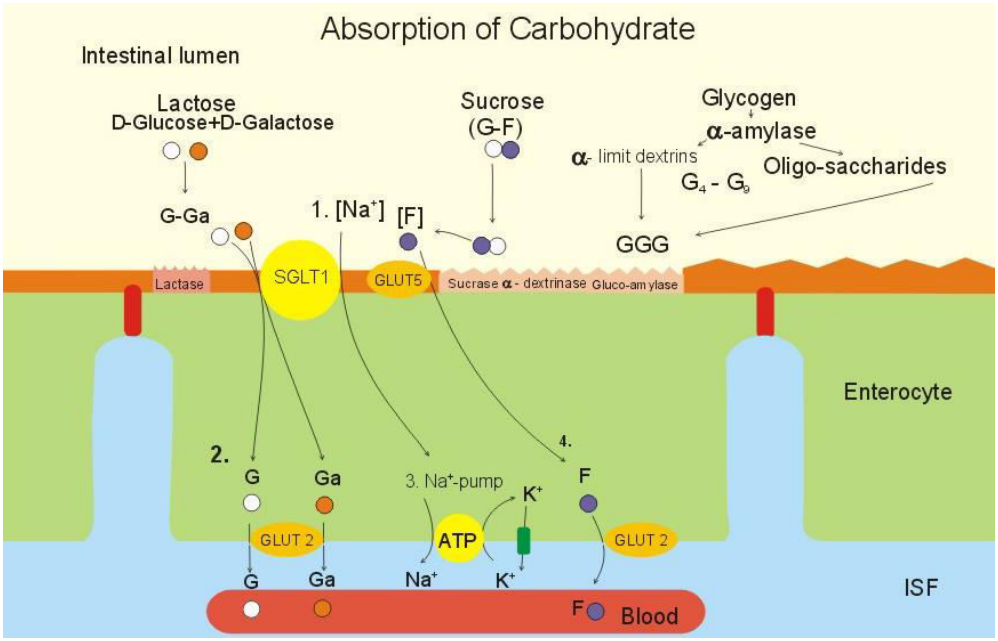


Figure 3.4 Absorption of carbohydrates by the enterocyte (Paulev & Zubieta-Calleja, 2012)

d. Gastrointestinal hormone response

During the period following the food ingestion, the digestion and absorption of nutrients is frequently associated with increased secretion of multiple gut peptides that act on distant target sites to promote the efficient uptake and storage of energy. The synthesis of these peptide hormones occurs in the enteroendocrine cells located in the epithelium of the stomach, small bowel, and large bowel. During the fasting state, these hormones are secreted at low basal levels (Drucker, 2007).

Incretin hormones causing an increase in the amount of insulin released from β cells of the islets (glucose-dependent insulinotropic polypeptide "GIP" and glucagon-like peptide-1 "GLP1") augment the magnitude of meal-stimulated insulin secretion from islet β cells in a glucose-dependent manner (Figure 3.5) (Drucker, 2006).

Native bioactive GLP1 rapidly lowers plasma glucose in patients with T2D, and a 3-weeks trial of pre-prandial subcutaneous GLP1 injections improved postprandial glycemic control and reduced levels of glucagon in the plasma of subjects with T2D. Furthermore, a modest weight loss with an improved insulin sensitivity were associated to the improved β cell function, and lowered fasting and postprandial glucose and HbA1c levels observed after continuous subcutaneous GLP1 infusion for 6 weeks (Zander *et al.*, 2002).

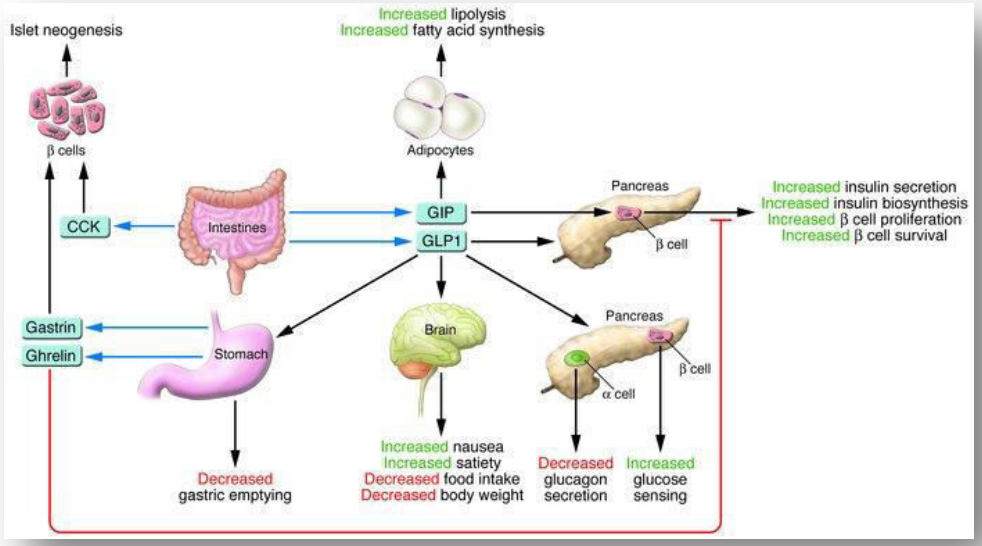


Figure 3.5 Actions of selected peptides on key tissues for the control of glucose homeostasis (Drucker, 2007)

Insulin biosynthesis, insulin secretion, and islet β cell survival are promoted by both GLP1 and GIP. GLP1 exerts important actions for regulation of glucose homeostasis, including inhibition of glucagon secretion and gastric emptying, and induction of satiety. However, GIP, but not GLP1, directly engages receptors on adipocytes coupled to energy storage. In contrast, CCK and gastrin might be important for stimulating the formation of new β cells by stimulating islet neogenesis but not for regulating levels of plasma glucose.

e. Pancreatic hormone response

The pancreatic polypeptides family of regulatory peptides gathers the two hormones, pancreatic polypeptides and peptide YY, and also the neurotransmitter, neuropeptide Y. After food intake, the endocrine F cells located in the periphery of the pancreatic islets produce the pancreatic polypeptides hormone by cholinergic mechanism (Ekblad & Sundler, 2002).

In response to a meal, plasma pancreatic polypeptides hormone concentrations increase in parallel with insulin. According to Schmidt *et al.* (2005), In relation to meal intake, the endocrine pancreatico-gastric response of pancreatic polypeptides may have impact as a metabolic counter regulation of glucose disposal. Pancreatic polypeptide delays the postprandial rise in plasma insulin and prolongs the glucose increment by inhibiting gastric emptying of solid meals.

3.2.2 Pathophysiology of postprandial hyperglycemia

3.2.2.1 Early decrease in insulin secretion

In response to glucose challenge, the insulin secretion is biphasic in nature. The first-phase response composed of an early burst of insulin release within 10 min and the second-phase response characterized by a gradual increase of insulin secretion that can last for several hours.

The second phase insulin secretion in conjunction with insulin sensitivity, in subjects with impaired glucose tolerance, influence the glucose tolerance test after 2-h (Van Haeften *et al.*, 2000). Further, in subjects with newly presenting T2D first-phase insulin secretion failed to correlate with postprandial glucose excursions during a meal tolerance test.

The second-phase insulin secretion during the hyperglycaemic glucose clamps was correlated with postprandial glucose excursions. When first-phase insulin secretion is lost, which comes along with a reduction of second-phase insulin secretion, primarily insulin sensitivity has an impact on postprandial glycemia-in subjects in an early stage of T2D (Rave *et al.,* 2010).

3.2.2.2 Increased insulin resistance

The link between T2D and insulin resistance has been recognized for more than fifty years. Insulin resistance, as a therapeutic target, is very important once hyperglycemia is present. It is also the most powerful predictor of future development of T2D. Increasingly, it is becoming recognized that postprandial hyperglycemia, for which insulin resistance is a chief determinant, has a very important role in determining cardiovascular disease risk, specifically in the occurrence of microvascular and macrovascular complications. According to recent epidemiological studies, and comparing to fasting glucose, the postprandial hyperglycemia was a greater risk factor for the risks of morbidity and mortality in T2D (Gao *et al.*, 2004). Furthermore, reductions in HbA1c do not necessarily indicate a reduction in postprandial hyperglycemia, which may explain why the reductions in HbA1c did not significantly reduce cardiovascular disease risk in recent studies (Yamagishi *et al.*, 2007).

3.2.2.3 Postprandial hyperglycemia, microvascular and macrovascular complications of diabetes

a. Impact of postprandial hyperglycemia on HbA1c

In T2D patients, HbA1c is more correlated with early post-meal/ late post- meal glucose levels than the fasting glucose levels (Avignon *et al.*, 1997). These early and late post-lunch glucose values are predictive for poor diabetes control, indicating that postprandial glucose is a major determinant in overall glycemic control.

The postprandial levels of glycemia play a disproportionate role in the development of both microvascular and macrovascular complications of diabetes, these complications can go undetected if only HbA1c values are assessed (Meigs *et al.*, 2002).

Self-monitoring technology is becoming an important element in the daily management of T2D. Since it allows easily measuring both fasting and postprandial glucose levels, and it provides patients and clinicians with relevant information on diurnal glucose fluctuations.

b. Postprandial glycemia and macrovascular complications

Levitan and colleagues (2004) performed a meta-analysis of 38 prospective studies and confirmed that hyperglycemia in the non-diabetic range was associated with increased risk of fatal and non-fatal cardiovascular disease, with a similar relationship between events and fasting or two-hour plasma glucose. In the analysis, 12 studies reporting fasting plasma glucose levels and six studies reporting postchallenge glucose allowed for dose-response curve estimates.

Cardiovascular events increased in a linear fashion without a threshold for two-hour post-meal plasma glucose, whereas fasting plasma glucose showed a possible threshold effect at 5.5 mmol/l (99 mg/dl). Similarly, in the Baltimore Longitudinal Study of Aging, Sorkin *et al.*, 2005 followed 1236 men for a mean of 13.4 years to determine the relationship between fasting plasma glucose and two-hour post-meal plasma glucose and all-cause mortality, all-cause mortality increased significantly above a fasting plasma glucose of 6.1 mmol/l (110 mg/dl) but not at lower fasting plasma glucose levels. However, risk increased significantly at two-hour post-meal plasma glucose levels above 7.8 mmol/l (140 mg/dl).

The observations also extend to people with diabetes with post-meal plasma glucose being a stronger predictor of cardiovascular events than fasting plasma glucose in T2D, particularly in women.

Relatively, few studies have analysed post-meal hyperglycemia as a risk factor in CVD development. In the Diabetes Intervention Study, in T2D patients who were monitored for 11 years, post-breakfast glucose levels, rather than fasting glucose, were related to myocardial infarction and death (Hanefeld *et al.*, 1996). Cavalot *et al.* (2011) add further evidence of the harmful relationship between post-meal glucose levels and CVD events.

c. Postprandial glycemia and microvascular complications

The uncontrolled glycemic peaks inducing over productions of superoxide activates four major pathways of hyperglycaemic damage to the tissues: the polyol pathway, advanced glycation end products formation, activation of protein kinase C isoforms and the hexosamine pathway (Brownlee, 2005). The activity of protein kinase C (isoform β) impairs contraction of smooth muscle cells or pericytes, increases production of basement membrane materials and enhances cell proliferation and capillary permeability. Thus, activation of protein kinase C-β by postprandial hyperglycemia could be responsible by microvascular complications that may be developing even in the early stages of diabetes (Koya & King, 1998). According to Vinik (2005), although macrovascular complications, such as myocardial infarction, stroke and gangrene, are only partially attributable to hyperglycemia and its attendant effect, the microvascular complications including retinopathy, nephropathy and neuropathy are directly related to the degree of hyperglycemia.

Data from the NHANES III showed that patients who had 2-hour postprandial glucose levels of 194 mg/dl had a three-fold increase in incidence of retinopathy, despite normal fasting glucose levels (fasting plasma glucose < 110 mg/dl, at the time of the study).

Studies of Pima Indian and Egyptian populations revealed a similar increase in the incidence of retinopathy in subjects with normal fasting glucose levels (< 110 mg/dl), but 2-hour postprandial glucose values of > 200 mg/dl (ADA, 2014).

d. Postprandial glycemia and oxidative stress

Ceriello (2005) investigated the role of postprandial hyperglycemia in the generation of oxidative stress and demonstrated that the production of free radicals was increased during the postprandial period and that this increase was proportional to the magnitude of the postprandial glucose excursions.

For instance, fasting nitrotyrosine, a metabolite derived from nitrosamine stress, was significantly increased in the diabetic patients. An additional increase was observed during post-meal periods. Reduction of the post-meal glucose excursions by using a pre-meal bolus of rapid insulin analog (aspart) resulted in parallel decrements in glycemic and nitrotyrosine responses. Such results have been confirmed by other studies (Sampson *et al.*, 2002).

3.2.3 Treatments of postprandial hyperglycemia

The treatment of post-prandial hyperglycemia (PPHG) begins with recognition of the overwhelming role of carbohydrate ingestion. Dietary management is more than mere reduction of caloric intake. The degree of post-prandial glucose elevation depends primarily on the carbohydrate content of the meal, and PPG and HBA1c levels can be lowered by reduction of the carbohydrate load (Kelley, 2003).

3.2.3.1 Diet and food composition

Carbohydrates are the most determinant dietary component in postprandial glycemic excursion. Other macronutrients can also influence the glycemic excursion, such as dietary

fibre content and fats, which decrease glucose absorption. The postprandial glycemia depends on the amount of carbohydrates ingested in grams as well as on the type of the carbohydrates in the diet (glycemic index) (Wolever & Bolgnesi, 1996).

A meal such as white bread and jelly with a glycemic index of 80 will result in a 2-fold higher incremental increase in glucose compared with an isocaloric meal of whole-grain bread and peanut butter with a glycemic index of 40. Most studies show that diets rich in high-glycemic-index, low-fiber foods independently increase the risk of both CV disease and T2D (Lichtenstein *et al.*, 2006).

Minimally processed plants such as vegetables, fruits, nuts, seeds, and grains generally increase post-prandial glucose PPG and triglycerides to a lesser degree than do processed foods. Ideal carbohydrate foods for improving post-prandial dysmetabolism include green leafy vegetables such as broccoli and spinach, or fruits such as grapefruits and cherries. Their lower caloric density and glycemic indexes and higher fibre and water content induce less glucose excursion after a meal, whereas their antioxidant phytonutrients dampen down the oxidant stress that is inherently generated when glucose or fatty acids are burned in the Krebs cycle (Mitrou *et al.*, 2007). The restriction of refined carbohydrates will improve the post-prandial levels of both glucose and triglycerides and can reduce intra-abdominal fat, particularly in individuals with insulin resistance.

Dietary fibres are effective at delaying gastric emptying, slowing digestion, and reducing post-prandial excursions of both glucose and triglycerides (Ma *et al.*, 2006). Minimally processed plant-based foods are natural sources of soluble and insoluble fibre that improve post-prandial dysmetabolism, reduce oxidant stress and inflammation, and lower the risks of coronary artery disease (CAD) and diabetes (Lichtenstein *et al.*, 2006; Ma *et al.*, 2006).

3.2.3.2 Pharmaceutical agents

Although most agents are classified by mechanism of action–enhancement of insulin secretion (sulfonylureas, nateglinide, and repaglinide) or delayed absorption of carbohydrates from the gastrointestinal tract (α-glucosidase inhibitors) individual agents vary in terms of their effects on fasting and postprandial hyperglycemia. All available antihyperglycemic agents reduce HbA1c level; however, few individual agents have been shown to specifically lower both fasting plasma glucose and postprandial plasma glucose concentrations. This may be a consequence of their mechanisms of action or of the trial design in that most studies have not systematically evaluated postprandial plasma glucose as an end point. Individual agents that have been specifically shown to target postprandial hyperglycemia in clinical studies include short-acting insulin analogues, α-glucosidase inhibitors, the short-acting insulin secretagogues (nateglinide and repaglinide), and glyburide–metformin tablets. Among the agents that lower postprandial plasma glucose level, repaglinide and glyburide–metformin tablets also lower fasting plasma glucose level effectively. Other agents lower fasting plasma glucose concentration significantly only when used in combination with another drug (Abrahamson, 2004).

3.3 Metabolism of Postprandial Lipemia

3.3.1 Factors influencing the postprandial lipid response

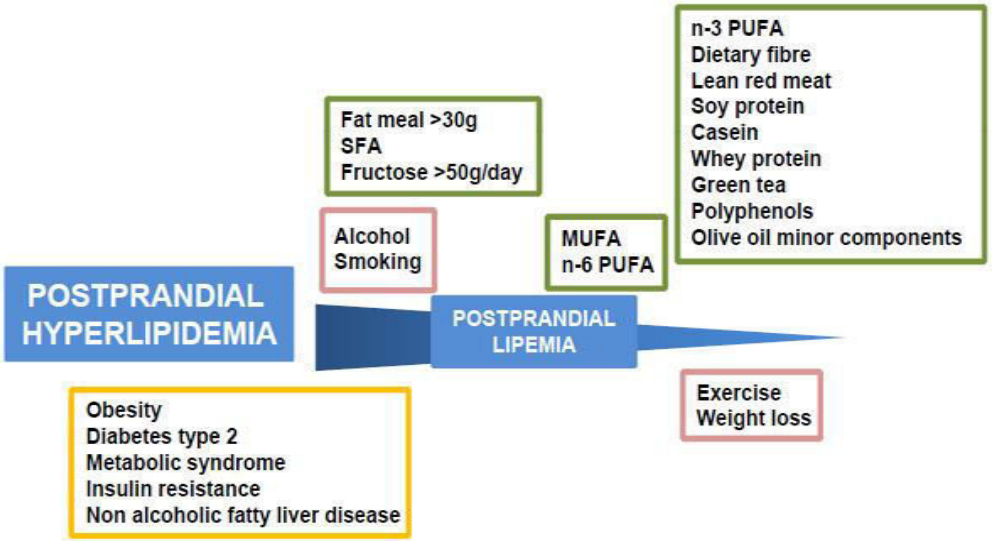


Figure 3.6 Lifestyle factors and metabolic diseases affecting postprandial lipemia (Bravo *et al.*, 2010)

3.3.1.1 Diet

Fat meals containing > 30g fat have been shown to cause postprandial lipemia, with dose dependence up to about 80g (Figure 3.6) (Lopez-Miranda *et al.*, 2007; Lairon, 2008). Comparing this to the average content of 20-40g fat in Western style meals and a typical dietary habit of 3-4 meals/day leads to the conclusion that postprandial triglyceridemia commonly lasts for 18h/day in the population of developed countries.

Acute studies testing the effects of single meals supplemented with different types of fats have shown that n-3 polyunsaturated fatty acids (PUFA) from fish oil attenuate the postprandial rise in blood TG levels (Lopez-Miranda *et al.*, 2007; Lairon, 2008) compared to other types of fat, and the saturated fatty acid (SFA) stearic acid found in animal fat has also been reported to have this effect (Berry *et al.*, 2007). In addition, changes in triglyceride-rich

lipoproteins (TRL) particle size and number and lipid and apolipoprotein (apo) composition depending on the type of fat in the meal have been demonstrated, with SFA causing the most pronounced lipemia by these criteria, followed by monounsaturated fatty acids (MUFA) (high in olive oil) and then PUFA (Lopez-Miranda *et al.*, 2007).

The carbohydrates content of the diet is also believed to influence postprandial lipemia (Lairon *et al.*, 2007). Although there is no consistent evidence to suggest that glucose has any effect, dietary fructose, when given both as the monosaccharide and in the disaccharide, sucrose (sugar), has been shown to enhance the response in a number of studies (Lopez-Miranda *et al.*, 2007; Lairon *et al.*, 2007).

Furthermore, there is some limited evidence to suggest that dietary protein quality influences postprandial lipemia. Diets containing lean red meat, soy protein or casein have been shown to reduce postprandial TRL levels (Lopez-Miranda *et al.*, 2007; Lairon, 2008).

3.3.1.2 Age

In general, tolerance to oral fat intake decreases with age. According to Issa *et al.* (2005), the differentiated behaviour of the postprandial lipemia in narrow and early age group intervals (20 to 30 years; 31 to 40 years; 41 to 50 years) is worth noting. Although the magnitude of postprandial triglyceridemia was inversely correlated with HDL-c levels and positively correlated with age and fasting levels of plasma TG. Information on postprandial lipemia in children is sparse, although in a recent study fasting TG and HDL-c, but not LDL-c levels, predicted the postprandial response. Interestingly, there was a significant difference in postprandial response between children and their mothers in spite of similar baseline TG levels (Couch *et al.*, 2000). There also appears to be an age-related change in postprandial lipemia and lipoprotein lipase (LpL) activity (Jackson *et al.*, 2003), which may in part be attributable to weight gain.

3.3.1.3 Lifestyle conditions

a. Physical activity

An acute bout of aerobic exercise has been shown to significantly reduce postprandial lipemia by 24–35 % and to significantly increase LpL activity (Zhang *et al.*, 2002).

The mitigation of the lipemic response to a meal high in fat and carbohydrate is related to the intensity and/or energy expenditure of the preceding exercise. Physical activity within the 24 h preceding a high-fat meal greatly improves the rate at which lipids are removed from the circulation. Furthermore, the postprandial response to an oral fat load is lower, and the clearance rates of TRL are higher, in endurance-trained individuals compared with untrained control subjects, although this may not be applicable to moderate exercise (Silvestre *et al.*, 2008). In a recent article, the combination of exercise and n-3 PUFA supplementation reduced postprandial lipemia response, measured as the incremental area under the postprandial curve of TG, to a greater degree in recreationally active males when compared with the two treatments individually (Smith *et al.*, 2004).

b. Smoking

Axelson *et al.* (1995) showed a 50 % greater TG postprandial increase in habitual smokers without changes in fasting TG. Smoking raised retinyl esters and apo B_{48} (by 170 %), but not apo B_{100}. Data obtained, in a large sample of men and women, support the interpretation of Axelson *et al.* (1995) that smoking affects postprandial TG metabolism. This was explained by primarily raising lipoproteins of intestinal origin since cigarette smokers had substantially greater postprandial retinyl palmitate and apo B_{48} (by 114–259 %) responses than did non-smokers, when adjusted for fasting triglycerides (Sharrett *et al.*, 2001).

c. Alcohol consumption

The effect of alcohol consumption on postprandial lipids has drawn continued attention over the past few years. Clearly, ethanol consumed with a meal elevates the very low-density lipoprotein (VLDL)-TG. In the study of Fielding *et al.* (2000), the addition of 47.5g alcohol to a high-fat meal (54 % of energy) was associated with an approximately 60 % increase in the peak plasma TG concentration compared with a meal consumed without alcohol. The authors attributed this increase to a stimulation of large VLDL secretion. Ethanol has also been shown to increase fatty acid synthesis and also to reduce TG clearance from the plasma (Pownall *et al.*, 1999).

3.3.1.4 Gender

Cox-York *et al.* (2013) observed a significantly lower (postprandial triglycerides) PPTG response in women than men, as has been reported by Horton *et al.* (2002). What is novel about their results is that the sex difference was driven by the significantly lower PPTG response of metabolic syndrome (MetS) women compared to MetS men.

A recent study reported that 8h after a high fat meal, MetS men had a significantly higher TG concentration than MetS women, which the authors suggest is likely due to differences in TG clearance (Adiels *et al.*, 2008). The women began to separate from the men at 6h post-meal. The reduction in TGs in MetS women is preceded by a significant increase in insulin with the breakfast and lunch meals, consistent with their reduced insulin sensitivity relative to the MetS men and normal weight subjects. The higher insulin excursion could have resulted in an increase in lipoprotein lipase (LpL) activity (Pedrini *et al.*, 2006) and increased TG uptake by tissues.

3.3.1.5 Genetic and postprandial lipemia

a. Polymorphisms in the apo A1/C3/A4/A5 gene cluster

Apolipoprotein A-1 (apo A1) is a key player in the reverse cholesterol transport, as apo A1 is the main protein component of HDL. However, apo A1 may also regulate postprandial lipemia, as suggested by the increased lipemic response described in previous studies (Perez-Martinez *et al.*, 2010). The relationship between apolipoprotein C-3 (apo C3) gene variations and postprandial metabolism is well stated. Apo C3 inhibits LpL, and, hereby, plasma apo C3 concentrations are positively associated with TG concentrations (Perez-Martinez *et al.*, 2008).

Apolipoprotein A-4 (apo A4) regulates dietary fat absorption and chylomicron synthesis, activates lecithincholesterol acyltransferase, and modulates LpL activation by apolipoprotein C-2 (apo C2). All these functions make easy to understand that gene variations in this locus can alter postprandial lipemia.

Apolipoprotein A-5 (apo A5) gene variations are clearly linked to altered postprandial TG metabolism (Szalai *et al.*, 2004).

b. Cholesterol ester transfer protein (CETP)

Cholesterol ester transfer protein (CETP) plays a significant role in HDL metabolism and reverse cholesterol transport. The magnitude of postprandial lipemia has been associated with plasma CETP concentration and lipoprotein content and size (Perez-Martinez *et al.*, 2010).

c. Glucokinase regulatory protein (GCKR)

In liver and pancreatic islet cells, glucokinase regulatory protein (GCKR) regulates glucokinase, which functions as a glucose sensor responsible for glucose phosphorylation in the first step of glycolysis. The current evidences supports the notion that significant predictive value from genetic markers can be gained by combining genotype information from multiple loci, such as GCKR and apo A5, on postprandial lipoprotein metabolism.

d. Hepatic lipase (HL)

Hepatic lipase (HL) has been implicated in the removal of remnant lipoproteins. Recently new data indicate that the presence of the A allele in the −250G/A promoter polymorphism of the HL gene is associated with a higher postprandial lipemic response (Jiménez-Gómez *et al.*, 2008).

e. Interleukin-6 (IL-6)

IL-6 is a major mediator of immune response and inflammatory processes. Shen *et al.* (2008) have demonstrated that the functional polymorphism −174C/G at the IL6 locus determines differences in both fasting and postprandial TG metabolism.

3.3.2 Measurement of postprandial lipids

3.3.2.1 Postprandial lipoproteins

Traditionally, the measurement of cholesterol associated with LDL has been used as an indicator of risk developing CHD in populations. LDL-c is routinely calculated in the post-absorptive state by the use of simple enzymatic-colorimetric tests. The measurement of chylomicrons and their remnants, however, is not a simple and usually involve lengthy and laborious postprandial studies. More recently however, techniques to assess post-absorptive

chylomicron remnant concentration and catabolism have been developed to allow simpler methods to predict the extent of postprandial lipemia.

3.3.2.2 Postprandial triglycerides

One of the classical postprandial markers of chylomicrons and their remnants is plasma triglycerides concentrations (Parks, 2001). Measurement of postprandial plasma triglyceride is the easiest method employed, usually involving an enzymatic colorimetric assay. At baseline, triglyceride levels are low and then increase steadily after meal with a peak usually at 3-4 hours, followed by a drop in levels. The initial rise in triglyceride concentration represents the packaging of increasing amounts of lipid into chylomicron particles and the decrease is the lipolysis of the triglyceride to the remnant particle (Mamo, 1995). Measurement of triglycerides provides general information on chylomicron metabolism as it is a combination of secretion, lipolysis and possibly clearance. It is not specific for intestinal lipoproteins, as it may also represent triglyceride packaging into VLDL. Lipoprotein species can be separated by ultracentrifugation, but a pure chylomicron or chylomicron remnant fraction cannot be isolated as the densities of intestinal lipoproteins can vary and overlap with other lipoprotein species (Mamo *et al.*, 1998).

3.3.2.3 Postprandial cholesterolemia

The limited data available indicate that important doses of dietary cholesterol (280 or 700mg per meal) tend to exacerbate the postprandial increase in triglyceridemia and especially chylomicrons, while a moderate dose (140 mg) does not generate noticeable change after a single meal (Dubois *et al.*, 1994). Nevertheless, the fact that ingested dietary cholesterol occurs in plasma chylomicrons during three subsequent postprandial periods (Beaumier-Gallon *et al.*, 2001) raises questions about the overall influence of dietary cholesterol on postprandial lipid metabolism. In view of the variable effects of cholesterol on

postprandial lipemia and possible prolonged duration of absorption, the question of standarization of meal cholesterol content, as well as intake in days preceding the tests meal, is an important consideration. In addition, habitual intake of plants sterols may need to be accounted for (Lairon *et al.*, 2007).

3.3.3 Pathophysiology of postprandial lipemia

3.3.3.1 Relationship between postprandial lipemia and cardiovascular diseases

The relationship between postprandial lipemia and cardiovascular disease has been recently strengthened by the results of two large prospective studies. In a Danish general population cohort of 7587 women and 6394 men, during a mean follow-up of 26 years, elevated nonfasting triglyceride levels predicted the occurrence of myocardial infarction, ischemic heart disease, and total death (Nordestgaard *et al.*, 2007). This effect was evident in both sexes, albeit more markedly in women, and was independent of the other cardiovascular risk factors measured.

A second prospective study in the United States evaluated initially healthy women (20 118 fasting and 6391 nonfasting) for a median of 11.4 years. After adjusting for age, blood pressure, smoking, and use of hormone therapy, both fasting and nonfasting triglyceride levels predicted cardiovascular events (nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, and cardiovascular death). Further adjustment for total cholesterol and HDL-c and measures of insulin resistance weakened this association among fasting participants, whereas nonfasting TG levels maintained a strong independent relationship with cardiovascular events. TG levels measured 2 to 4 hours after a meal had the strongest association with cardiovascular events, with the association progressively weakening with longer fasting (Bansal *et al.*, 2007).

These two large prospective studies with a huge number of cardiovascular events provide the strongest available evidence of the role of postprandial lipemia in the occurrence of cardiovascular disease.

3.3.3.2 Postprandial lipemia and atherosclerosis

In the event that the liver is overproducing VLDL particles, like in central obesity, the metabolic syndrome, T2D (Alipour *et al.*, 2007), the common catabolic steps for VLDL and chylomicrons become saturated resulting in accumulation of both VLDL and chylomicron remnants. According to the classical concept of atherosclerosis by postprandial lipemia, remnant lipoproteins penetrate the vessel wall and are taken up by monocytes inducing foam cell formation. This may be one of the first steps in atherogenesis.

Postprandial and remnant lipoproteins may induce the expression of leukocyte adhesion molecules on the endothelium, facilitating recruitment of inflammatory cells. Activation of only endothelial cells is not sufficient to initiate the process of atherogenesis. Leukocyte activation and binding to the endothelium are obligatory steps (Ross, 1999; Alipour *et al.*, 2007). A cytokine-controlled sequential up-regulation of selectins and adhesion molecules on activated leukocytes and endothelial cells is necessary. It has been shown that neutrophils increase postprandially with concomitant production of pro-inflammatory cytokines and oxidative stress and that these changes may contribute to endothelial dysfunction (Van Oostrom *et al.*, 2006). In healthy volunteers and in patients with premature atherosclerosis, postprandial lipemia has been associated with the up-regulation of leukocyte activation markers. Fasting leukocytes of patients with CVD have an increased lipid content when compared to controls and it was suggested that this was due to the uptake of chylomicrons in the bloodstream (Tertov *et al.*, 1992). Recently, we have shown that apo B binds to neutrophils and monocytes and that postprandially, leukocytes

become enriched with meal-derived fatty acids suggesting a direct interaction between lipoproteins and leukocytes by the leukocytes' uptake of exogenous fatty acids in the bloodstream. Increased residence time of atherogenic lipoproteins in plasma will result in enhanced binding of these particles to the endothelium thereby creating a marginated pool of endothelial bound lipoproteins (Verseyden *et al.*, 2004), increasing the level of activation of the endothelial cells which potentially will expose more adhesion molecules on their surface (Alipour *et al.*, 2007). These series of events will ultimately lead to the adhesion of inflammatory cells (especially, monocytes and lymphocytes but potentially also neutrophils) to the activated endothelium. Therefore, we have hypothesized that atherogenesis may start in the bloodstream and not in the sub-endothelium as generally considered.

3.3.3.3 Postprandial hyperlipidemia during insulin resistance and type 2 diabetes

T2D is characterized by elevated fasting triglycerides due to increased levels of VLDL, low HDL and the presence of small dense LDL. Increasing evidence arising from the investigation of postprandial lipemia in T2D has added postprandial dyslipidemia as another feature of diabetic dyslipidemia. There are a number of clinical studies reporting postprandial dyslipidemia in T2D.

Previous investigations using triglycerides as a marker have found an elevation in postprandial triglyceride response in hypertriglyceridemia (Mero *et al.*, 2000) and normotriglyceridemic T2D subject. The postprandial triglyceride response has also been found to be independently associated with the progression of atherosclerosis despite normal fasting triglyceride levels in T2D (Teno *et al.*, 2000).

Postprandial studies using retinyl palmitate as a marker for intestinal lipoproteins have also found an elevation in the postprandial responses (De Man *et al.*, 1996). Previous investigations using semiquantitative determinations of $apoB_{48}$ found elevations in fasting and

postprandial levels of apoB_{48} (Mero *et al.*, 2000). These studies showed an association between raised fasting triglycerides and increased levels of apoB_{48} in T2D. They also suggested an increase in chylomicron synthesis as a possible cause of postprandial dyslipidaemia in combination with delayed clearance. The fasting marker of intestinal lipoproteins; remnant-like particle-cholesterol (RLP-c), also has been found to be elevated in T2D in both the cholesterol and triglyceride measure fractions (Watanabe *et al.*, 1999). The other fasting marker for impaired TRL metabolism, apo C3 has been found to be an independent marker of cardiovascular risk in T2D (Gervaise *et al.*, 2000).

Collectively, these studies suggest impairment in the metabolism of intestinal lipoproteins at both fasting and postprandially in T2D and the dyslipidemia may contribute to the presence of CAD in T2D.

3.3.4 Treatment of postprandial lipemia

3.3.4.1 Diet and life style changes

Lifestyle modifications in diet, physical activity and smoking habits are the major therapeutic components of primary prevention of CHD (Kromhout *et al.*, 2002). A change in diet is the first and preferred therapeutic measure for postprandial dyslipidemia as it is: inexpensive, can be easily incorporated into daily life, has no potential side effects and so safe to continue over a lifetime, may reduce dyslipidemia without the use of drugs, but if used in combination, can also augment the effect of lipid-lowering drugs. The main dietary changes recommended by the NCEP ATP III are a reduction in the intake of saturated fats and cholesterol, using therapeutic dietary options for enhancing LDL lowering (e.g. plant stanols/sterols) and weight reduction (Gotto, 2002).

Saturated fats have been found to elicit impaired postprandial lipoprotein metabolism (Thomsen *et al.*, 1999). Replacement of saturated fats with monounsaturated or

polyunsaturated fats, in particular *n*-3 fatty acids (fish oils) which have been found to lead to a reduction in postprandial lipemia (Park & Harris, 2003). Other possible dietary supplements that could improve postprandial lipemia are plant sterols and antioxidant vitamins (Relas *et al.*, 2000). Flavonoids and catechins supplementation in nuts, vegetables, fruits and seeds and tea have also been found to be modulators of lipid levels. The effects on postprandial lipemia however are unknown.

Other lifestyle changes include an increase in regular aerobic physical activity and a cessation in smoking. Aerobic exercise has been shown to reduce postprandial lipemia as well as triglyceride and LDL-c levels and increase HDL-c and LpL activity (Gill *et al.*, 2001; Kromhout *et al.*, 2002). In combination with modification in diet and weight loss, the effects are increased. Cessation in cigarette smoking is also recommended as smokers have been found to have impaired postprandial lipoprotein metabolism in comparison to non-smokers (Watts *et al.*, 1998).

3.3.4.2 Pharmacotherapy

Pharmacology is the next option for postprandial dyslipidemia if dietary therapy and lifestyle changes are ineffective. The main therapeutic options are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), fibrates, and nicotinic acid derivatives. Other therapeutic agents are hormone replacement therapy (HRT), resins and T2D, hypoglycaemic. Statins are however, the most commonly used therapeutic agent for lipid-lowering as they have been shown to be safe and effective in lowering LDL-c levels and as well as triglyceride levels (Ginsberg, 1998).

3.4 Relationship between Lipids and Glucose Metabolism

3.4.1 Physiological effects of fatty acids on insulin secretion

In obese and diabetic subjects, insulin resistance leads to inability to suppress lipolysis and lipid oxidation. These subjects manifest high lipid oxidation during insulin-stimulated conditions (Felber *et al.*, 1987), despite low rates of lipid oxidation in fasting conditions, the latter being a key mechanism responsible for excess lipid accumulation within skeletal muscle, which in turn contributes to insulin-resistant glucose metabolism, mainly through substrate competition processes (Kelley & Mandarino, 2000). Moreover, high levels of circulating fatty acids (FAs) enhance the expression of fatty acid translocase and fatty acid transport protein within the muscle cell.

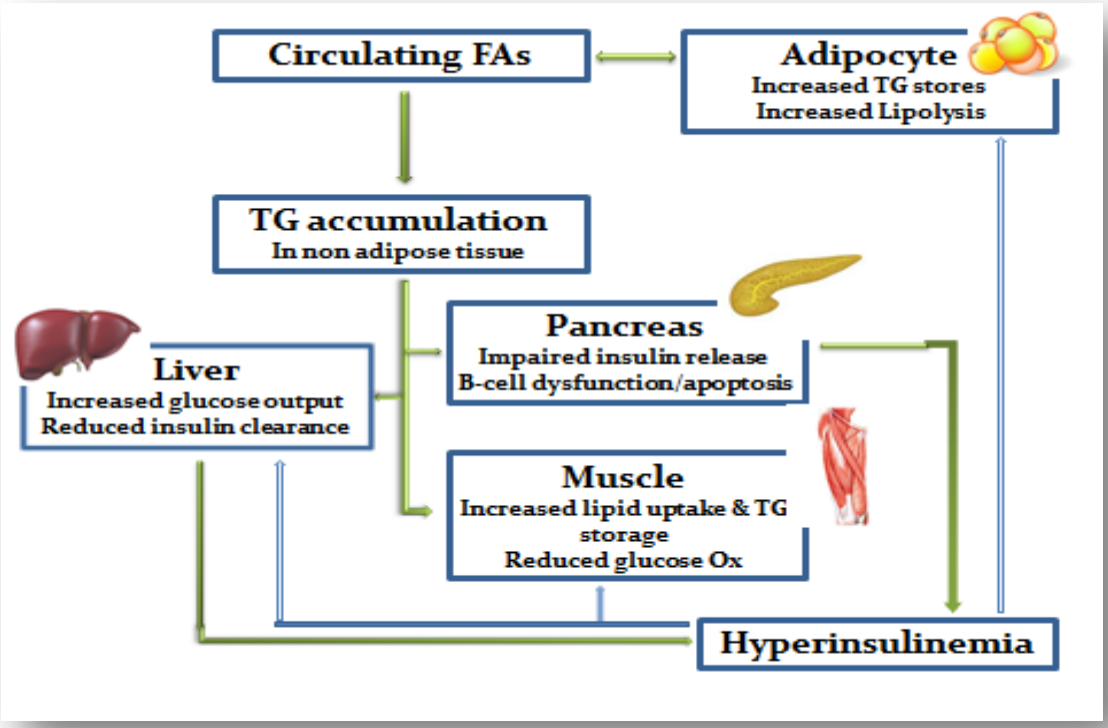


Figure 3.7 Triglycerides accumulation in tissues (Manco *et al.*, 2004)

Excessive intake of FAs leads to an accumulation of triglycerides in many tissues (Figure 3.7), particularly in the adipose tissue, in which lipolysis is increased by a mass effect. This, associated with the development of adipocyte insulin resistance, results in a net spillover of FAs to non-adipose tissues, such as muscle, liver and pancreas. In insulin-resistant subjects, increased levels of tissue FA-binding and transport proteins in adipose and non-adipose tissues facilitate the uptake processes. In skeletal muscle, the increased lipid oxidation may contribute, in part, to the glucose oxidation and non-oxidative glucose uptake deficiencies. In the pancreas, prolonged exposure to FAs might cause exhaustion of insulin release through the mechanism of lipotoxicity. In the liver, FAs promote gluconeogenesis and reduce insulin clearance (Manco *et al.*, 2004).

The equilibrium between oxidation and re-esterification is of paramount importance in determining FA storage in tissue, and, recently, a great deal attention has been focused on the content, localization and composition of fat within skeletal muscle as determinants of insulin resistance. Several studies reported an inverse relationship between the action of insulin and muscle TG content in obese and diabetic subjects (Pan *et al.*, 1997), as well as between the action of insulin and the degree of saturation in subjects with a wide range of BMIs (Manco *et al.*, 2000). Muscle attenuation on computed tomography scans, which should reflect the intramuscular lipid content, has been reported to be accentuated in obese women and to be reciprocally related to insulin sensitivity. Proton magnetic resonance spectroscopy, a validated method for the measurement of lipid depots in muscle tissue, has demonstrated a reciprocal relationship between intramyocellular lipid accumulation and insulin sensitivity in healthy subjects (Szczepaniak *et al.*, 1999). In obese subjects, the fat depletion of intramyocellular TG depots that occurs after bariatric surgery is strictly related to an improvement in insulin sensitivity, independently of the degree of weight and fat mass loss (Greco *et al.*, 2002).

3.4.2 Inhibition of pancreatic beta cells function by fatty acids

High-fat feeding in rodents consistently impairs insulin stimulated glucose uptake in both fat and muscle, the main tissue determining insulin-stimulated glucose uptake. Furthermore, high-fat and high-fat–high-protein diets are associated with a greater incidence of diabetes in non-obese diabetic mice (Jacqueminet *et al.*, 2000).

Fatty acids may cause β-cell death, at least in islets of Zucker diabetic fatty rats. These rats constitute an extremely obese genetic model of T2D bearing a mutation in the leptin receptor gene. In Zucker diabetic fatty rats, β-cell dysfunction is related to increased triacylglycerol content in islets (Unger, 1997), which leads to increased production of nitric oxide, ceramide synthesis and b-cell apoptosis.

Exposure for prolonged periods of time to high levels of palmitate and glucose, however, also stimulates the synthesis of neutral lipids in normal β-cells while causing simultaneous inhibition of insulin gene expression.

Part Two: Experimental Research

CHAPTER 4

PATIENTS &METHODS

CHAPTER 4

PATIENTS &METHODS

4.1 Objective of the Study

The objective of the study was to show the main role of measuring biochemical parameters in overweight/obese patients with T2D, of both genders, to compare these parameters between the fasting and the postprandial stages and to assess whether those responses are associated with each other.

4.2 Study Design

The study design performed in this thesis was chosen to maximally test the hypothesis stated in our objective section. The investigation involves comparing over fasting and postprandial periods a group of diabetic overweight or obese patients with two other groups as control; overweight or obese individuals without diabetes and non-obese ones with T2D using a prospective multicenter case-control study.

4.2.1 Study location

This study took place in two cities located in the North-Western region of Algeria;

- Sidi-Bel-Abbes city: Public Establishment of Local Health Centre (Diabetes Centre of Ex Gambetta) and Mostefa Ben Brahim polyclinic

- Mascara city: Meslem Tayeb Hospital.

4.2.2 Study duration

The experimental protocol of the study lasted for almost 33 months, from November 2011 to July 2014;

- *First stage (November 2011 -Mai 2012):* **Establishment of the experimental protocol**

- Preparation of questionnaires, clinical plugs and food diaries necessary for the conduct of the investigation in accordance with the objectives set for three groups of patients;

- Acquisition of necessary reagents;

- Reviewing of patient records at the level of the three health facilities in which the study was conducted.

- *Second stage (October 2012 -February 2014):* **First assessment**

- This first evaluation was conducted in overweight and obese diabetic patients and normal weight diabetic patients at the level of three health facilities.

- *Third Stage (March 2014 - July 2014):* **Second assessment**

- Carrying out the study in non-diabetic overweight and obese individuals.

- *Fourth stage (August 2014 -December 2014):* **Analysis of the study data**

4.3 Patients

The investigations into postprandial metabolism are conducted in three groups of patients. Described below are the inclusion and exclusion criteria for each patients group.

- *Overweight and obese diabetic patients:*

The inclusion criteria were:

- Male and female with T2D;

- Aged 18-75 years;

- Overweight or obese; BMI $\geq$ 25 kg/m^2;

- Following an oral and/or dietary treatment;

- Free from any degenerative health complication.

• *Normal weight diabetic patients:*

The inclusion criteria were the same as the previous group except for BMI (<25kg/m^2).

• *Non-diabetic overweight and obese individuals:*

The selection criteria included:

- Male and female;
- Aged 18-75 years;
- Overweight or obese; BMI ≥ 25 kg/m^2;
- Free from any health complication.

Exclusion criteria were used to reduce confounding effects of other factors that might have an impact on the postprandial metabolism; hypothyroidism, primary hyperlipidemia, pregnancy and all other confirmed health complications were absent in the three groups.

4.3.1 Recruitment of subjects

In the three health departments, diabetic patients meeting the inclusion criteria were asked to participate in the study. Agreement from patients, patient's general practitioner and/or physician, to join the trial was obtained. After that, we explained to our patients through face to face meeting sessions, the aims of the investigation, procedures, time commitments and all queries about the trial. Finally, we discussed the modalities of filling questionnaires and food diaries.

The same stages were performed with non-diabetic volunteers who gave their agreements to participate in the study.

4.4 Anthropometric Measurements

Body weight (in kilograms) was measured using an electronic balance (TS-2003A: 360 Ib, Capacity: 180 Kg, Graduations 0.1Kg) and height (in meters) was measured with a

body meter (Seca 206, Germany; Measuring range: 0 – 220 cm, Graduation Length: 1 mm) which measured to the top of the head of a shoeless patient.

The BMI was calculated as follow: BMI (kg/m^2) = weight (kg)/height^2 (m^2). Patients should be lightly dressed and they must respect the appropriate position for height measurement (gathered feet, straight body, heels touching the wall and staring out the horizon).

Waist circumference was measured respecting every single cm with a measuring tape (Maximum: 150 cm, Graduation Length: 1 mm), the tape is gently tightened around the patient's abdomen roughly in line with the navel without depressing the skin.

4.5 Blood Pressure Measurement

OMRON *M3* Digital Automatic Blood Pressure Monitor (Omron Healthcare., Ltd. Kyoto, Japan) was used for calculating the morning blood pressure.The machine can determine systolic blood pressures between 30–240 mmHg, diastolic 10–210 mmHg and heart rate 40–200 beats per minute. Patients were quietly in a supine position for blood pressure readings, with three readings taken over a 5–minute period. The average of the three readings is taken as the blood pressure.

4.6 Blood Sampling and Assay Methods

The blood samples were carried out in conditions ensuring the reliability of results; each patient remained in a supine position while a 21–gauge needle was inserted into an antecubital forearm vein by a trained nurse. Blood was then collected into vacutainer collection tubes containing heparin anticoagulant.

For fasting glucose and lipid profiles, venous blood samples were collected from each patient 12 hours after an overnight fast. We did not get information about the kind of food taken before 12 hours of blood testing. However, for the postprandial state, blood samples were drawn 2 hours after a breakfast meal for glucose and 3–4h for lipid parameters. The usual breakfast meal in almost 94% of our patients provides an average of 692.0 ± 11.03 kcal (fat: 44.1±0.4g [57%], protein: 7.6±0.11g [6%], carbohydrates 49.4±0.71g [38%]).

4.6.1 Assessment of carbohydrate metabolism

4.6.1.1 Glucose was determined by enzymatic colorimetric method (GOD-POD (see annex files). Liquid). (SPINREACT-Spain *ISO 9001 certified*)

4.6.1.2 HbA1c levels were determined by ion exchange chromatography. The hemolysate is deposited onto a column filled with negatively charged resin. First eluted fast hemoglobins: HbA1a, HbA1b, HbA1c and the main fraction HbA0. The percentage of the different fractions is determined by spectrophotometric measurement.

4.6.2 Exploration of lipid metabolism

The following parameters have been evaluated too

4.6.2.1 Total Cholesterol (TC): Serum cholesterol was measured using an enzymatic colorimetric method (CHOD-POD. Liquid)(SPINREACT-Spain *ISO 9001 certified*), the following biochemical reactions were involved in this assay:

$$\text{Cholesterol esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{4-Cholestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{POD} \text{Quinonimine} + 4H_2O$$

The amount of pink quinoneimine dye produced is stoichiometrically related to the concentration of total free cholesterol. The dye can be detected spectrophotometrically at 500nm.

4.6.2.2 High-density lipoprotein cholesterol (HDL-c) using a precipitating reagent (SPINREACT-Spain *ISO 9001 certified*).

4.6.2.3 Low-density lipoprotein cholesterol (LDL-c) with direct determination of serum LDL-c levels without the need for any pre-treatment or centrifugation steps (Cholesterol LDL Enzymatic colorimetric. Liquid- A-I. SPINREACT-Spain *ISO 9001 certified*) (see annex 1).

4.6.2.4 Triglycerides (TG) measurement of serum triglycerides were determined by enzymatic method (Triglycerides GPO-POD. Enzymatic colorimetric of SPINREACT-Spain *ISO 9001 certified*) (see annex 1).

4.6.2.5 Apolipoproteins A-I (apoA-I) is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. apo A-I is a cofactor for lecithin-cholesterol-acyl-transferase, the enzyme responsible for forming cholesteryl esters in plasma and plays an important role in the transport of cholesterol from peripheral tissues to the liver, to be finally excreted.

Turbidimetric test for the measurement of apolipoprotein A-I in human serum or plasma was used (see annex 1).

Anti- apo A-I antibodies when mixed with samples containing apo A-I, form insoluble complexes. These complexes cause an absorbance change, dependent upon the apo A-I concentration of the patient sample, that can be quantified by absorbance measurement (at 600 nm) and by comparison from a calibrator of known apo A-I concentration. (SPINREACT-Spain *ISO 9001 certified*).

4.6.2.6 Apolipoproteins B (apo B) is the major structural apolipoprotein in VLDL (Very Low Density Lipids), LDL (Low Density Lipids) lipoproteins and chylomicron. The most important function is the transport of rich tryglicerides lipoproteins from liver and intestine to other tissues.

Turbidimetric test for the measurement of apolipoprotein B in human serum or plasma was employed (see annex 1).

Anti- apo B antibodies when mixed with samples containing apo B, form insoluble complexes. These complexes cause an absorbance change, dependent upon the apo B concentration of the patient sample that can be quantified by absorbance measurement (at 340 nm) and by comparison from a calibrator of known apo B concentration. (SPINREACT-Spain *ISO 9001 certified*).

4.7 Questionnaires Distribution

Our pre-constructed questionnaire consisted of a number of questions developed according to our objectives. The cooperation of physicians and nutritionists in the development of our questionnaire was an important step.

The questionnaire entitled "*Diabetes, Obesity & Postprandial Metabolism*" (see annex 2) included the five following parts:

4.7.1 Personal data

Patient identification through; name, age, gender, address, telephone and E-mail.

4.7.2 Socioeconomic data

Data related to marital status, level of study, occupation, type of housing and residence.

4.7.3 Lifestyle information

The history of diabetes, obesity, heredity, age of the disease, knowledge and attitude towards different aspects of diabetes (for diabetic patients) has been addressed to our patients too.

4.7.4 Food and hygiene behaviour

Attitude of patients towards diet, physical exercises and the follow up was assessed by using some open ended questions.

4.7.5 Questions about RAMADAN fasting

This section provides some information, from the investigate patients, about the practice of fasting during the holy month and their general behaviours.

4.8 Checking Patient Records

The reviewing of medical records of diabetic patients was highly considered to get extra information which could not be previously obtained through questionnaires, such as:

- The evolution of their body weight;
- The changes in biological parameters;
- The duration of their diabetes;
- Risk factor recordings: history of cigarette smoking, hypertension, hyperlipidemia, and for women, the use of oral contraceptives and hormone replacement therapy;
- Recorded measurements of blood pressure;
- Medication.

4.9 Food Intake Assessment

The most accurate heavy protocols are obtained by direct weighing of consumed foods using a balance and the weighing of the leftover food. This technique requires from respondent cooperation and an important investment that may enhance the appearance of certain biases in the recording method.

However, food records method, called food diaries, requires that the subject (or observer) reports all foods and beverages consumed during a specified period (usually one to seven days). Amounts of each food item may be recorded. If nutrient intakes are to be calculated, the amounts consumed should be estimated as accurately as possible. Amounts may be determined by weighing or by estimating volumes. In some situations, only those foods of particular interest are recorded.

We have opted to the food records method; all patients (diabetic and/or obese) completed a three-day food diary administered at screening. Patients were given verbal and written instructions on recording their food and drinks, which included the recording of the type of food, time of meal, serving size and method of cooking and other details. For patients who were unable to fill out their food diaries alone we asked from a family member to fill out the diaries for them.

For the amount units of consumed food, we asked the surveyed patients to use some standard units such as : tea or coffee spoon, slice (thin or thick), cup, glass (juice glass, water glass), corner for cheese, bag, bottle, can, packet, middle or normal piece, meat ball and standard portion for fruits and cakes.

For food products packaged in small unit such as dairy products, cakes and small tin cans, we asked patients to indicate commercial brand of the product.

A thorough verification step following the filling of both questionnaires and food records was organized with every patient individually to correct inaccurate data and oversights.

4.10 Statistical Analysis

All data were processed and analyzed through SPSS 20.0 (*Statistical Package for the Social Sciences*, IBM Corporation; Chicago, IL August 2011) for Windows (SPSS, 2011). For all analyses, a *p*-value of 0.05 or less was considered to be significant. The statistical techniques used in this thesis are described in this section.

4.10.1 Standard deviation

Standard deviation (SD) measures variation in values of a given group of samples around their mean and is used throughout this thesis. The SD is calculated by taking the square root of the variance.

$$SD = [\sum (xi - \mu)^2 / n-1]^{1/2}$$

xi: sample value

μ: population mean

n: population size

4.10.2 Distribution of data

Assessment of the normality of data distribution is the initial step of all statistical analysis conducted in our thesis. Normality was determined using Kolmogorov-Smirnov's test.

4.10.3 Independent samples *t*–test

Most of the comparisons conducted in this work are between two independent groups (male and female). The independent *t*–test compares the means of a variable between two gender groups. The independent *t*–test is calculated by the following equation:

$t = (\mu 1 - \mu 2) \text{ SE } (\mu 1 - \mu 2)$

$\mu 1$ and $\mu 2$: the means of the two populations

SE: the standard error of the mean difference between the groups

4.10.4 Paired samples *t*–test

The paired samples *t*–test compares the means of two variables for a single group (e.g. between fasting and postprandial states). It calculates the differences between the values of two variables for a case and tests whether that differs from 0. Paired *t*–test is calculated by the following equation:

$t = [d - 0] / \text{SE } (d)$ where d is the observed mean difference.

4.10.5 Correlation

Correlation analysis or a correlation coefficient is a statistic that describes the strength and direction of the relationship between two variables. A correlation coefficient ranges from –1 to +1. A positive correlation is a relationship in which both variables increase together and a negative is one in which one variable increases while the other decreases.

4.10.6 Linear regression

Linear regression allows the prediction of the value of a dependant variable (e.g. y) with the value of an independent variable (e.g. x). In regression it is assumed that a change in x will directly lead to a change in y. This can be expressed in a regression equation as:

$y = a + bx$ where *a* is the intercept and *b* is the regression coefficient or slope

4.10.7 Analysis of variance

Analysis of variance (ANOVA) allows the comparison of several observations in a single analysis. It is based on a model of the data which assumes that any particular value X_{ij} can be accounted for by summing a number of components.

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Xij: the jth replicate from treatment I

μ: the total mean

αi: the effect of treatment

εij: the error associated with Xij

4.10.8 Non–parametric tests

The Kruskal Wallis test is a popular nonparametric test to compare outcomes among more than two independent groups. It is used to compare medians among k comparison groups (k>2) and is sometimes described as an ANOVA with the data replaced by their ranks.

The test statistic for the Kriskal Wallis test is denoted H and is defined as follows:

$$H = \left(\frac{12}{N(N+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_j} \right) - 3(N+1)$$

K: the number of comparison groups

N: the total sample size

n_j: the sample size in the j^{th} group

R_j: the sum of the ranks in the j^{th} group

4.11 Calculation of Food Rations

The diaries were analysed using the software program NutriSurvey for windows 2007, SEAMEO-TROPMED RCCN-University of Indonesia (NutriSurvey, 2007). This program is based on the German food database (BLS) with English names in addition to other databases (USDA SR25 -2012-, FAO-Minilist worldwide and Egypt food database) and provides information on energy, fat, carbohydrate, protein, vitamins and minerals.

The main characteristics of this program are as follows:

- The German food database (BLS) includes 11000 foods with more than 130 nutrients;

- It contains all useful functions which are typical for this kind of software (nutrient analysis and calculation of energy requirements, planning of diets, diet history, food frequency, searching of nutrients in foods, handling of recipes,..);

- The possibility to analyze several food records together with a variety of options;

- The nutrient values of the foods in the database can easily be changed and complemented with additional information;

- Recipes can be modified and the nutrient content corrected depending on the preparation of the recipe.

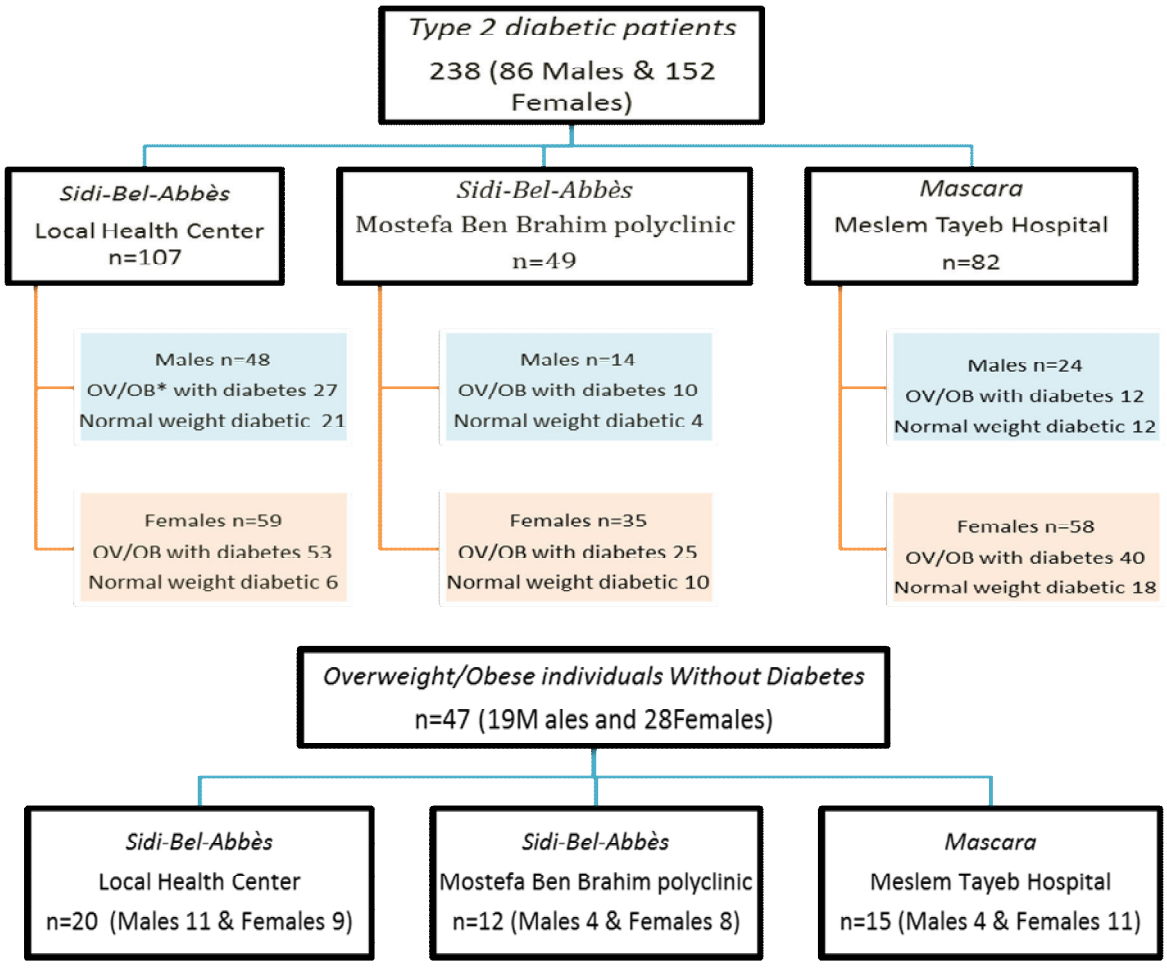
CHAPTER 5

RESULTS

CHAPTER 5

RESULTS

The current study aimed to compare over fasting and postprandial states a group of diabetic overweight/obese patients (n=167) with two other groups as control; overweight/obese individuals without diabetes (n=47) and normal weight patients with type 2 diabetes (n=71), using a prospective multicenter case-control study. 285 patients (105 males and 180 females) represent the three groups. The whole studied population was distributed in three health departments located in two cities of western Algeria as shown in figure 5.1:



*OV: Overweight, OB: Obese

Figure 5.1 Description of the studied population (n=285)

5.1 Anthropometric Assessments

Anthropometric characteristics of the three groups are presented in Tables 5.1 and 5.2

Table 5.1 Anthropometric characteristics of the three groups N=285

	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**							
Age (Years)	26.00	68.00	47.68±12.53	21.00	60.00	44.07±12.30	0.332
Weight (Kg)	81.00	110.00	94.26±9.20	73.00	103.00	89.53±8.94	0.086
Height (cm)	158.00	186.00	178.10±6.34	154.00	177.00	168.78±5.43	<0.001
Ideal Body Weight (Kg)	56.00	77.00	71.07±4.76	52.40	66.20	60.91±3.63	<0.001
Waist Circumference (cm)	86.00	107.00	100.44±5.49	87.00	105.00	98.160±5.12	0.152
BMI (Kg/m^2)	25.28	34.98	29.82±3.27	25.18	37.97	31.41±2.85	0.084
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**							
Age (Years)	19.00	75.00	57.02±11.65	26.00	75.00	58.25±10.17	0.497
Diabetes Duration (Years)	0.50	15.00	5.89±3.13	0.50	15.00	7.36±3.89	0.020
Weight (Kg)	64.00	110.00	87.75±10.93	58.00	113.00	76.97±11.55	<0.001
Height (cm)	156.00	186.00	171.95±6.93	146.00	178.00	160.01±6.86	<0.001
Ideal Body Weight (Kg)	54.50	77.00	66.16±5.10	47.60	85.40	56.23±4.92	<0.001
Waist Circumference (cm)	70.00	125.00	101.86±9.78	51.00	150.00	101.52±13.45	0.872
BMI (Kg/m^2)	25.06	39.92	29.73±3.87	25.10	44.14	29.96±4.00	0.732
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**							
Age (Years)	25.00	75.00	56.62±14.99	21.00	73.00	55.61±14.36	0.775
Diabetes Duration (Years)	2.00	15.00	6.90±3.67	1.00	15.00	6.17±3.84	0.417
Weight (Kg)	51.00	80.00	66.47±7.56	52.00	74.00	63.67±6.16	0.094
Height (cm)	154.00	184.00	170.64±7.13	153.00	175.00	163.97±5.99	<0.001
Ideal Body Weight (Kg)	53.00	75.50	64.96±5.26	51.80	65.00	58.37±3.60	<0.001
Waist Circumference (cm)	53.20	106.00	88.22±10.61	51.00	108.00	86.66±10.68	0.539
BMI (Kg/m^2)	19.20	24.97	22.76±1.57	20.83	24.98	23.63±1.15	0.010

(*)Significantly different at $p<0.05$, **Min**.; minimum, **Max**.; Maximum, **BMI**; Body Mass Index, **S.D**.; Standard Deviation, **M**; Male, **F**; Female

Table 5.2 Comparison between basic characteristics of the three groups

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal-Wallis test	
						Chi-squared value (X^2)	p value of asymptotic significance*
Age (Years)	50.00 45.53±12.38	$p<001$	58.00 57.88±10.60	$p=0.941$	60.00 56.14±14.60	32.148	<0.001
Diabetes Duration (Years)	00.00		6.00 6.93±3.74	$p=0.447$	6.00 6.55±3.75	118.525	<0.001
Weight (Kg)	91.00 91.44±9.25	$p<001$	80.00 80.14±12.36	$p<001$	67.00 65.13±7.02	115.970	<0.001
Height (cm)	173.00 172.55±7.38	$p<001$	164.00 163.51±8.76	$p<001$	168.00 167.45±7.37	41.574	<0.001
Ideal Body Weight (Kg)	63.80 65.02±6.48	$p<001$	59.00 59.14±6.71	$p<001$	61.40 61.81±5.60	31.510	<0.001
Waist Circumference (cm)	99.50 99.08±5.33	$p=0.053$	102.00 101.62±12.46	$p<001$	87.50 87.47±10.60	72.746	<0.001
BMI (Kg/m^2)	30.82 30.77±3.09	$p=0.045$	29.28 29.90±3.95	$p<001$	23.31 23.18±1.44	161.658	<0.001

(*)Significance of the test at $p<0.05$, **S.D.**; Standard Deviation, **BMI**; Body Mass Index

The mean age in patients of the three groups (**1**: overweight/obese individuals without diabetes, **2**: overweight/obese diabetic patients, and **3**: normal weight diabetic patients) was 55.41±12.77 years (55.19±13.42 in males and 55.54±12.40 in females). Diabetes duration was 5.69±4.25 years (5.18±3.92 in males and 5.99±4.41 in females).

Table 5.1 shows the anthropometric characteristics of male and female patients in each group. There were no significant gender differences in age, average of diabetes duration, waist circumference and BMI. However, highly significant differences were observed in the height and ideal body weight between males and females in the three groups.

Furthermore we noticed a highly significant difference ($p<0.001$) of body weight between women and men within the group of overweight/obese diabetic patients.

Table 5.2 shows the comparison between basic characteristics of the three groups of patients using ANOVA Kruskal-Wallis test (a non-parametric test). There were high significant differences ($p<0.001$) between the three groups of patients for the whole studied

parameters (age, duration of diabetes, weight, height, ideal body weight, waist circumference and BMI). The use of possible pairwise combinations between the main group (overweight/obese diabetic patients) and the two other groups as control disclosed no significant differences within the group of normal weight diabetic patients for age (p=0.941) and with overweight/obese non diabetic patients for waist circumference (p=0.053).

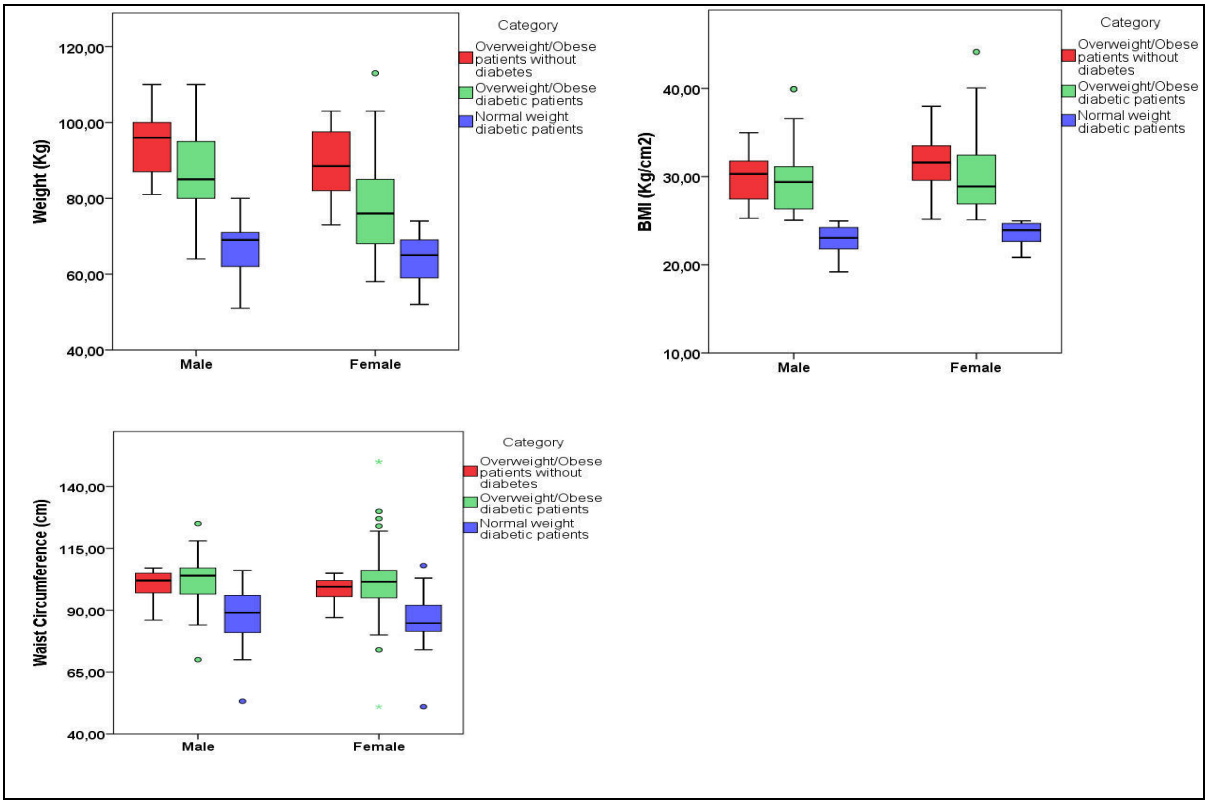


Figure 5.2 Comparison of body weight, BMI and WC within the three groups

We compared the body weight, BMI and waist circumference among the three groups of patients. Body weight and BMI were higher in non-diabetic overweight/obese individuals compared to overweight/obese diabetic patients of both genders. However, WC was higher in overweight/obese diabetic patients comparing to non-diabetic obese ones.

Comparing between the two genders, our results have revealed that males had often higher weight and WC within the three groups of patients. However, BMI was higher in overweight/obese non-diabetic women and normal weight diabetic women.

5.2 Clinical Assessment

Since T2D and hypertension are two diseases often associated, especially in obese subjects, measurement of blood pressure is an important clinical parameter for monitoring diabetic and/or obese patients. Hypertensive patients with diabetes or obesity are more predisposed to target organ damage, resulting stringent targets for blood pressure control.

The obtained results in our three patient groups about these parameters are summarized in table 5.3 and table 5.4

Table 5.3 Comparison of blood pressure values between males and female over patients group

	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)** **Goal blood pressure,** <140/90 mmHg [1]							
Systolic Pressure (mmHg)	120.0	150.0	126.7±8.30	110.0	150.0	127.5±11.0	0.795
Diastolic Pressure (mmHg)	60.0	89.0	79.9±8.30	60.0	90.0	76..5±9.6	0.221
Overweight/Obese Diabetic Patients (n=167, 49M and 118F**)** **Goal blood pressure,** <140/80 mmHg [2]							
Systolic Pressure (mmHg)	110.0	187.0	129.8±14.5	90.0	191.0	130.0±15.7	0.949
Diastolic Pressure (mmHg)	59.0	95.0	76.1±9.9	52.0	95.0	76.2±9.4	0.940
Normal Weight Diabetic Patients (n=71, 37M and 34F**)** **Goal blood pressure,** <140/85 mmHg [1]							
Systolic Pressure (mmHg)	90.0	191.0	127.1±17.5	110.0	151.0	125.5±11.6	0.647
Diastolic Pressure (mmHg)	52.0	90.0	76.1±8.9	55.0	91.0	74.4±10.1	0.454

(*)Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **S.D.**; Standard Deviation, **M**; Male, **F**; Female.

[1] according to the American Diabetes Association (ADA, 2013). [2] according to the European Society of Cardiology & the European Society of Hypertension (Mancia *et al.*, 2013).

Table 5.4 Comparison of blood pressure values between the three groups

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal-Wallis test	
						Chi-squared value (X^2)	*p* value of asymptotic significance*
Systolic Pressure (mmHg)	124.0 127.2±9.9	*p*=0.629	126.0 129.9±15.3	*p*=0.055	120.0 126.3±14.9	4.082	0.130
Diastolic Pressure (mmHg)	80.0 77.9±9.2	*p*=0.267	80.0 76.1±9.5	*p*=0.383	79.0 75.3±9.4	2.727	0.256

(*)Significance of the test at $p<0.05$, **S.D.**; Standard Deviation

No significant differences ($p = 0.05$) were found between the two genders concerning the blood pressure values as reported in table 5.3.

Using the ANOVA Kruskal-Wallis test (Table 5.4), there were neither differences between the three groups of patients nor between each pairs combination. Nevertheless, obtained averages measurements are at the limit of recommended values proving that our patients were not very high-risk groups. Similarly, extremes peaks were noted in several patients of the three groups.

5.3 Analysis of Questionnaires Results

5.3.1 Socio-professional data

5.3.1.1 Marital status

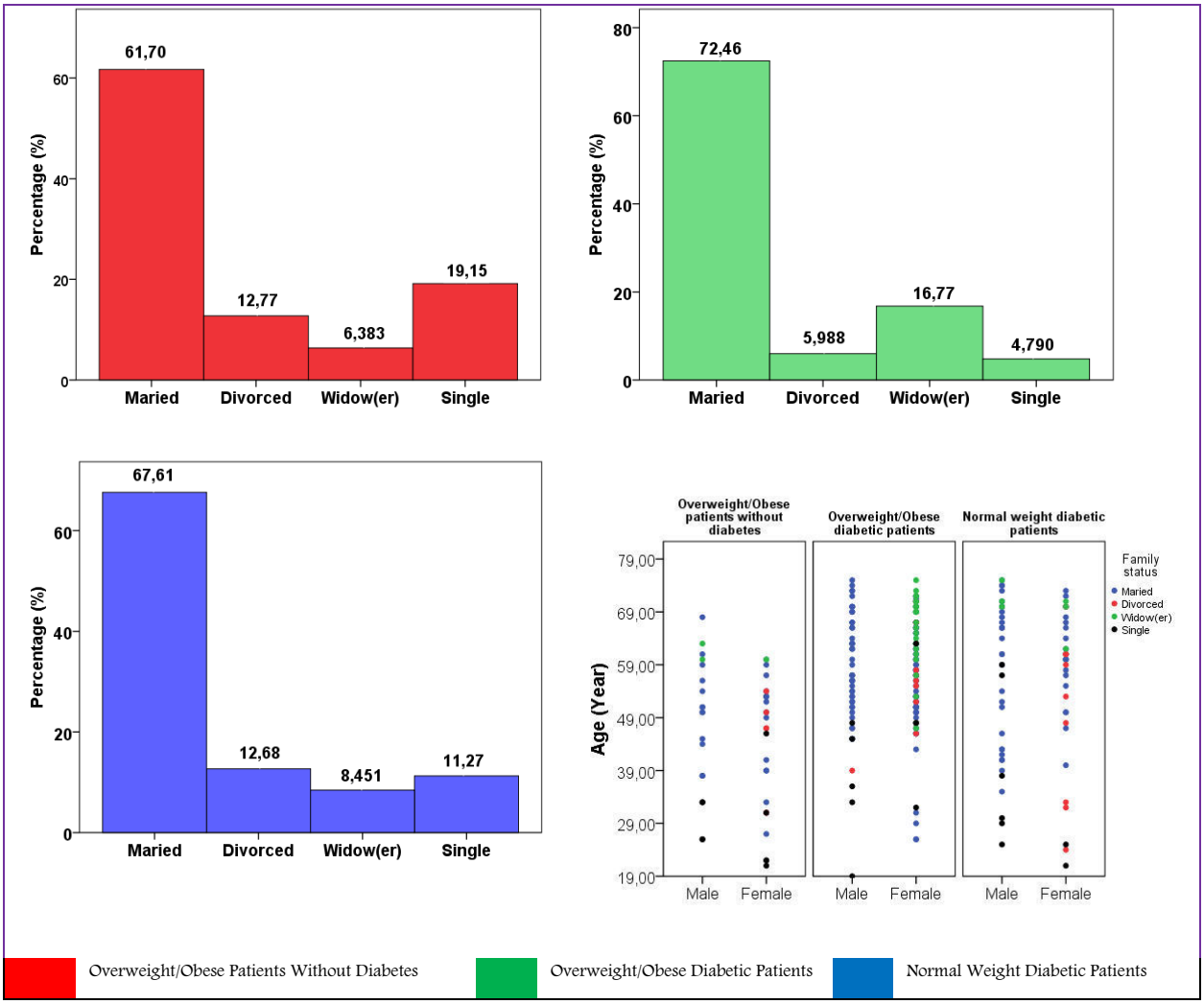


Figure 5.3 Percentage distributions of patients according to their marital status

The census of marital status in our patients indicated that the class of "Married" was the most represented in the three groups of patients. This class is followed by "Singles" (19.15%) in overweight/obese patients without diabetes, by "Widow(er)" in overweight/obese diabetic patients (16.77%) and by "Divorced" in normal weight diabetic patients (12.68%).

The study of marital status under each group of patients, according to the age and the gender, showed that "single" class was more related to younger age (< 50 years). The class of "widow (er)" was more encountered in older individuals (≥ 50 years). However, the two classes of "Married" and "Divorced" were more likely to be represented by all age categories for both genders.

5.3.1.2 Educational level

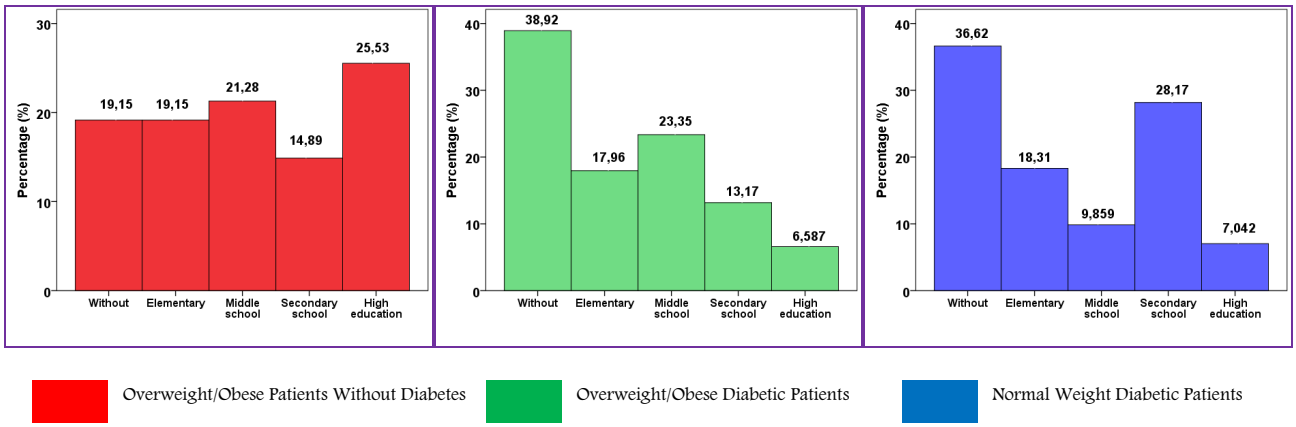


Figure 5.4 Percentage distributions of patients according to their education level

The intellectual level plays an essential role for a diabetic patient who wants to prevent or to reduce the disease complications.

Analyses of questionnaires revealed that over a third of diabetic (normal weight, overweight or obese) patients were illiterates. Those with a high education level (university) represented only about 7% of patients in both groups. However, patients who completed at least the middle and/or secondary school represented a considerable proportion among our patients in these two groups.

For the group of non-diabetic overweight/obese patients, more than 25% reported having a university level, 21.28% have a middle school level, and 19.15% have an elementary school level with the same percentage (19.15%) for the illiterates.

5.3.1.3 Occupational status

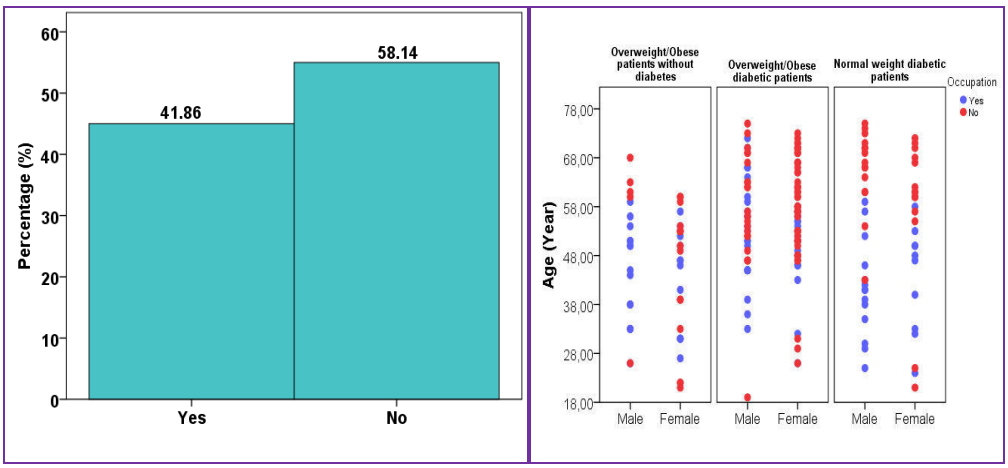


Figure 5.5 Relationship between professional activity, age and gender according to groups of patients

The professional activity data, for the whole sample, indicated that over 41% of all patients still exerted a function, while 58% reported not to have a professional activity or are jobless. The age of 60 years remains generally the limit to perform different professional activities by our patients of both genders with a small male dominance over female in the three groups.

These results did not reflect the financial situation of our patients, since in the category of unemployed are also classified retirees, household women, students and beneficiaries of different social pensions.

5.3.2 Assessment of physical activity and sports

Table 5.5 Assessment of sport practiced by each patients' group

		Gender		Chi-squared test		
		Male	Female	Chi-squared value (X^2)	*p* value*	Cramér's V
Overweight/Obese patients without diabetes	Academic Sports	-	7.69%	7.37	0.025	0.62
	Soccer	9.61%	1.92%			
	Walking	11.53%	5.76%			
Overweight/Obese diabetic patients	Academic Sports	-	-	2.21	0.137	0.37
	Soccer	11.53%	-			
	Walking	13.46%	5.76%			
Normal weight diabetic patients	Academic Sports	1.92%	3.84%	8.24	0.016	0.56
	Soccer	15.38%	-			
	Walking	3.84%	7.69%			

(*)Significance of the test at $p<0.05$

Since many decades, it is admitted that there is an inverse relationship between physical activity and weight gain. Likewise, exercise has always been considered a cornerstone of diabetes management, along with diet and medication.

Over our whole sample, 23.58% of patients practiced sport steadily of whom; 36.51% were non-diabetics overweight/obese, 30.75% were overweight/obese diabetics and 32.67% were normal weight diabetics.

The study of types, frequency of physical activity (per week) as well as the time spent for each workout session revealed the following results:

- Physical activities practiced by our patients were usually academic sports (Represented by collective games as running, jumping, basketball, handball and volleyball), Soccer and walking ;
- The weekly frequencies of physical activities practices varied from a group to another; 1.59±0.79 in non-diabetic overweight/obese patients, 2.41±1.54 in overweight/obese diabetics and 4.29±2.33 in normal weight diabetics;
- The time spent (in minutes) for each workout session was 30.59±9.98 in non-diabetic overweight/obese patients, 40.59±17.12 in overweight/obese diabetics, and 36.79±25.91 in normal weight diabetics;

- The type and the frequency of physical activities varied by age and gender of each patient;
- A significant difference ($p<0.05$) between males and females was found with regard to the type of physical activity in non-diabetics overweight/obese patients and normal weight diabetic ones.

5.3.3 Assessment of family antecedents

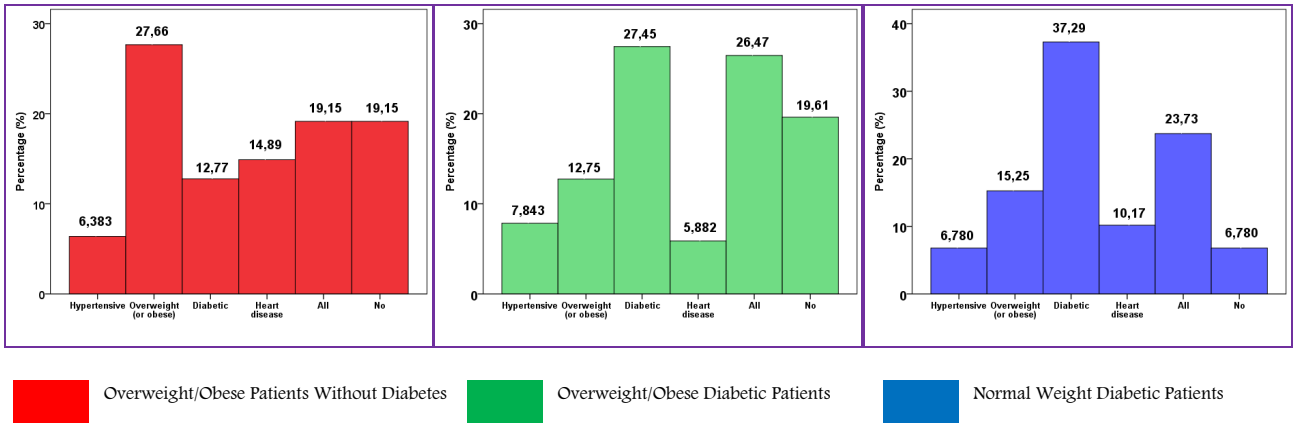


Figure 5.6 Assessment of family history in each patients group

The family history of diabetes, obesity, hypertension and heart diseases in parents and siblings was analysed for each group of patients. Results are shown in figure 5.5.

In non-diabetic overweight/obese patients, the frequency of a positive family history was above 27% for the weight excess, while the frequency of diabetes history was 12.77%. However, patients with no family history represented 19.15%.

For overweight/obese diabetic patients and normal weight diabetic patients the frequencies of a positive family history, regarding diabetes, were 27.45% and 37.29% respectively. However, frequencies of all diseases combined (diabetes, obesity, hypertension and heart diseases) were 26.47% and 23.73% respectively.

Table 5.6 Comparison of family history by gender in each patients group

		Gender		Chi-squared test		
		Male	Female	Chi-squared value (X^2)	*p* value*	Cramér's V
Overweight/Obese patients without diabetes	Hypertensive	-	1.44%	5.57	0.34	0.34
	Overweight/obese	1.92%	4.32%			
	Diabetic	0.96%	1.92%			
	Heart disease	2.40%	0.96%			
	All	1.92%	2.40%			
	No	1.92%	2.40%			
Overweight/Obese diabetic patients	Hypertensive	0.96%	2.88%	2.65	0.75	0.16
	Overweight/obese	2.88%	3.36%			
	Diabetic	4.80%	4.65%			
	Heart disease	0.48%	2.40%			
	All	5.28%	7.69%			
	No	4.32%	5.28%			
Normal weight diabetic patients	Hypertensive	0.96%	0.96%	1.80	0.87	0.10
	Overweight/obese	2.88%	1.44%			
	Diabetic	5.28%	5.28%			
	Heart disease	0.96%	1.92%			
	All	3.84%	2.88%			
	No	0.96%	0.96%			

(*)Significance of the test at $p<0.05$

The comparison between the two genders, within the three groups of patients, indicated that no significant differences existed between women and men according to their family history, neither for positive nor negative associations.

5.4 Analysis of Food Diaries

5.4.1 Total energy intake

Table 5.7 Comparison of total energy intake and energy fulfilment between men and women in each group of patients

	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**							
Total Energy Intake (Kcal)	1757.50	2541.20	2190.44±271.13	1346.50	2313.30	1864.92±409.28	0.064
Overweight/Obese Diabetic Patients (n=167, 49M and 118F**)**							
Total Energy Intake (Kcal)	1822.40	2465.90	2280.26±236.92	1877.30	2582.10	2167.90±233.94	0.381
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**							
Total Energy Intake (Kcal)	1537.00	2071.00	1856.01±182.06	1270.00	2420.60	1828.50±384.95	0.874

(*)Significantly different at $p<0.05$, **S.D.**; Standard Deviation

First, we calculated the daily meal frequency for all groups. The averages were 3.62±0.419 meals/day in the overweight/obese non-diabetic group, 3.18±0.385 meals/day in the overweight/obese diabetic group, and 3.42±0.604 meals/day in the normal weight diabetic group with no significant difference ($p> 0.05$) between them.

The comparison of total energy intake (TEI) revealed no significant differences ($p>0.05$) between men and women in the three groups of patients.

The highest values of total energy intake were recorded in the group of overweight/obese diabetic patients of both genders, followed by the group of overweight/obese non-diabetic patients, then by the group of normal weight diabetic patients.

5.4.2 Total energy intake according to daily meals

Table 5.8 Distribution of total energy intake in different daily meals among the three groups according to patient's gender

	Male			Female				*p* value for Student *t*-test*
	Min.	Max.	Means±S.D (%)	Min.	Max.	Means±S.D (%)	R.	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**								
Breakfast (Kcal)	88.20	373.30	200.73±97.81 (9.97%)	171.30	552.60	375.17±156.80 (20.14%)	20%	0.012
Morning Snack (Kcal)	0.00	0.00	0.00	0.00	0.00	0.00	10%	-
Lunch (Kcal)	700.50	1216.80	909.48±206.74 (41.25%)	504.10	925.90	664.48±125.06 (36.32%)	30%	0.008
Afternoon Snack (Kcal)	0.00	394.302	203.80±112.74 (9.53%)	0.00	394.40	141.00±130.97 (6.90%)	10%	0.292
Dinner (Kcal)	638.70	1060.60	876.42±150.87 (40.24%)	444.70	917.80	684.25±165.70 (36.66%)	30%	0.020
Overweight/Obese Diabetic Patients (n=167, 49M and 118F**)**								
Breakfast (Kcal)	158.90	481.90	333.40±146.78 (14.38%)	169.60	475.20	361.80±88.09 (16.88%)	20%	0.645
Morning Snack (Kcal)	0.00	342.50	90.23±135.18 (4.54%)	0.00	18.20	2.02±6.06 (0.10%)	10%	0.068
Lunch (Kcal)	534.90	1570.50	1060.11±349.8 (45.95%)	610.00	1265.80	969.47±214.65 (44.89%)	30%	0.542
Afternoon Snack (Kcal)	0.00	504.80	133.86±217.41 (5.77%)	0.00	478.80	209.20±193.21 (9.36%)	10%	0.493
Dinner (Kcal)	162.50	969.00	662.75±310.08 (29.35%)	175.70	893.60	625.41±225.02 (28.74%)	30%	0.790
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**								
Breakfast (Kcal)	158.90	394.30	231.06±92.03 (12.60%)	88.20	411.40	239.68±113.58 (13.56%)	20%	0.880
Morning Snack (Kcal)	0.00	0.00	0.00	0.00	0.00	0.00	10%	-
Lunch (Kcal)	534.70	1242.10	808.21±308.32 (42.81%)	421.20	1416.50	830.26±364.80 (44.28%)	30%	0.905
Afternoon Snack (Kcal)	0.00	338.10	175.71±150.03 (9.56%)	0.00	311.50	137.70±138.20 (7.58%)	10%	0.622
Dinner (Kcal)	503.10	884.00	641.01±138.28 (35.01%)	364.80	808.50	620.84±158.85 (34.57%)	30%	0.804

(*) Significance of the test at $p<0.05$, **S.D.**; Standard Deviation, **R.**: recommendation according to Lacroix & Assal, 2003.

The analysis of food diaries and the distribution of TEI according to the daily meals highlighted the following notes:

- The lunch was the most important daily meal that brought the highest amount of calorie intake for all patients;

- The calorie intake of lunch and dinner exceeded the needs in all patients of both genders. However, the breakfast calorie intakes were lower than the dietary recommendations;
- There were highly significant differences between males and females for breakfast, lunch and dinner calorie intakes in the group of overweight/obese non diabetic patients. While, no differences, were observed between men and women within the two groups of diabetic patients.

Table 5.9 Comparison of daily meals contribution in TEI between the three groups

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal-Wallis test	
						Chi-squared value (X^2)	*p* value of asymptotic significance*
Total Energy Intake (Kcal)	2149.70 2027.68±376.12	*p*=0.255	2195.40 2212.89±233.64	*p*=0.001	1868.20 1839.50±310.98	9.018	0.011
Breakfast (Kcal)	242.60 287.95±155.33	*p*=0.174	349.10 350.44±111.07	*p*=0.010	215.50 236.24±102.06	5.951	0.051
Morning Snack (Kcal)	0.00 00.00		0.00 37.30±92.46		0.00 00.00	--	--
Lunch (Kcal)	709.60 786.98±208.13	*p*=0.015	972.90 1005.73±268.61	*p*=0.085	704.60 821.44±331.84	5.827	0.054
Afternoon Snack (Kcal)	156.40 172.40±122.87	*p*=0.971	93.60 179.06±199.18	*p*=0.881	152.40 152.90±139.01	0.043	0.979
Dinner (Kcal)	770.65 780.33±182.77	*p*=0.181	636.10 640.34±252.25	*p*=0.663	608.30 628.91±146.13	4.911	0.086

(*)Significance of the test at $p<0.05$, **S.D.**; Standard Deviation

The comparison between our three groups of patients indicated a high significant difference in TEI and energy provided from breakfast between T2D patients with normal weight against those overweight/obese.

The caloric contribution of lunch was significantly different between non-diabetic individuals in overweight or obese against diabetic patients of the same weight class.

5.4.3 Assessment of main energy nutrients intake

Table 5.10 Distribution of the main energy nutrients by patient's gender in the three groups

	Male				Female				*p* value for Student *t*-test*
	Min.	Max.	Means±S.D (%)	R.	Min.	Max.	Means±S.D (%)	R.	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F)									
Carbohydrates (g)	172.90	326.50	228.17±50.79 (53.55%)	50-65%[1]	41.20	281.60	181.27±106.3 (46.35%)	50-65%[1]	0.250
Lipids (g)	54.20	148.40	93.40±33.45 (21.81%)	15-30%[2]	41.10	102.40	85.44±19.83 (26.89%)	15-30%[2]	0.548
Proteins (g)	85.00	139.20	103.37±20.38 (24.63%)	10-35%[1]	61.20	144.70	89.26±30.26 (26.75%)	10-35%[1]	0.263
Dietary Fibres (g)	19.20	35.00	26.72±6.48	≈ 38 g/d[3]	8.10	35.90	22.41±11.84	≈ 25 g/d[3]	0.352
Total water (g)	1643.00	3214.90	2644.23±540.20	--	1744.50	2815.70	2162.86±393.59	--	0.046
Overweight/Obese Diabetic Patients (n=167, 49M and 118F)									
Carbohydrates (g)	156.10	234.80	193.66±28.59 (46.65%)	45-65%[2]	150.30	305.40	219.33±51.30 (53.50%)	45-65%[2]	0.288
Lipids (g)	91.50	146.90	120.78±20.65 (29.10%)	15-30%[2]	84.00	136.00	105.20±19.35 (26.27%)	15-30%[2]	0.160
Proteins (g)	77.70	143.40	100.55±22.30 (24.23%)	10-20%[1]	45.60	119.60	82.11±22.07 (20.21%)	10-20%[1]	0.138
Dietary Fibres (g)	21.00	35.60	25.36±5.63	≈ 38 g/d[3]	17.80	29.40	23.58±4.65	≈ 25 g/d[3]	0.516
Total water (g)	1244.70	3328.80	2631.83±763.05	--	1190.20	3645.40	2474.08±897.99	--	0.730
Normal Weight Diabetic Patients (n=71, 37M and 34F)									
Carbohydrates (g)	176.70	258.00	209.31±27.68 (57.77%)	45-65%[2]	152.60	287.20	199.71±36.61 (56.81%)	45-65%[2]	0.596
Lipids (g)	49.50	94.80	75.45±16.83 (20.60%)	20-30%[4]	36.80	139.50	77.73±35.80 (21.47%)	20-30%[4]	0.883
Proteins (g)	47.80	135.30	79.70±31.40 (21.62%)	10-20%[1]	57.10	96.30	76.92±14.92 (21.71%)	10-20%[1]	0.820
Dietary Fibres (g)	15.10	40.50	26.21±8.67	≈ 38 g/d[3]	17.90	32.80	24.72±5.93	≈ 25 g/d[3]	0.697
Total water (g)	2335.70	4203.90	3144.08±713.02	--	2129.60	3370.10	2642.93±404.91	--	0.104

R.: Recommendations, (*) Significance of the test at $p<0.05$, **S.D.**; Standard Deviation, **g/d**: Grams/day

[1] According to Otten *et al.*, 2006. [2] according to Evert *et al.*, 2013. [3] according to NRC, 2005. [4] according to Elmadfa & Kornsteiner, 2009.

Assessment of dietary intakes of main energy nutrients showed no significant difference between men and women in the three groups of patients. However, a significant difference in total water quantity (in grams) was observed in overweight/obese non-diabetics group between the two genders (p=0.046).

We studied the average composition of the daily ration in each group (by patient's gender). Assessment results of carbohydrates, lipids, proteins and fibres are shown in Table 5.10.

***Carbohydrates**:* The carbohydrate intake was covered in, in both genders of the three patients groups. An exception was noticed in women overweight / obese non-diabetic (46.35% of TEI <50-65%).

***Total Lipids**:* Energy intake from total dietary fats, in all groups, was acceptable according to the macronutrient distribution range established by the FAO/WHO (Smit *et al.*, 2009).

***Proteins** :*Results about dietary intake showed slight increase in proteins intake, comparing to the requirements established by Otten *et al.,* (2006), in all diabetic patients either women or men, with overweight/obesity or not. However, results of overweight/obese non diabetic subjects were in the recommended ranges.

***Dietary Fibres**:* Different fibres have different properties and thus varying functions. They aid in laxation and promote satiety, which may help reduce energy intake and therefore the risk of obesity. They can also attenuate blood glucose levels, normalize serum cholesterol levels, and reduce the risk of cardiovascular diseases.

Table 5.11 Comparison between main energy nutrients consumed by the three patients group

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal–Wallis test Chi-squared value (X^2)	ANOVA Kruskal–Wallis test p value of asymptotic significance*
Carbohydrates (g)	222.95 204.72±84.38	p=0.426	204.60 209.06±44.33	p=0.901	198.00 203.55±32.64	1.421	0.491
Lipids (g)	90.65 89.42±26.99	p=0.019	103.00 111.43±20.70	p=0.001	71.10 76.88±28.94	11.716	0.003
Proteins (g)	90.25 96.32±26.06	p=0.492	88.00 89.48±23.31	p=0.101	76.80 78.03±21.95	4.734	0.094
Dietary Fibers (g)	27.50 24.56±9.52	p=0.871	23.80 24.30±4.95	p=0.836	25.30 25.32±6.89	0.046	0.977
Total water (g)	2400.05 2403.55±521.11	p=0.492	2645.90 2537.18±821.67	p=0.419	2764.56 2843.39±582.96	3.080	0.214

(*)Significance of the test at $p<0.05$, **S.D.**; Standard Deviation

The use of possible pairwise combinations between the main group (overweight/obese diabetic patients) and the two other groups as control had not disclosed any significant differences in main dietary energy nutrients. An exception was noted for lipids, where we noticed significant difference between the main group on the one hand, and normal weight diabetic patients (p=0.001) and overweight/obese non diabetic patients (p=0.019) on the other hand.

5.4.3.1 Assessment of carbohydrates intake

Carbohydrates are the major source of energy in the human diet. There are four types of carbohydrates; monosaccharaides, disaccharides, oligosaccharides, and polysaccharides (Gropper & Smith, 2013). The majority of the average human's carbohydrate intake consists of polysaccharides and simple sugars (monosaccharaides and disaccharides) (Gropper & Smith, 2013).

The following table shows the comparison of carbohydrates dietary intakes between women and men in the three groups of patients.

Table 5.12 Carbohydrates intakes of males and females in the three groups

	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**							
Monosaccharaides (g)	11.40	36.40	20.15±7.94	8.70	41.10	14.42±10.38	0.207
Disaccharides (g)	6.20	72.30	29.43±20.93	14.50	32.90	22.22±6.76	0.340
Polysaccharides (g)	118.40	270.90	176.56±45.32	16.00	239.60	141.37±97.14	0.339
Overweight/Obese Diabetic Patients (n=167, 49M and 118F**)**							
Monosaccharaides (g)	4.60	36.40	23.15±11.85	5.60	26.30	15.50±6.60	0.131
Disaccharides (g)	9.50	74.70	30.88±25.21	17.80	81.10	48.74±25.41	0.204
Polysaccharides (g)	103.80	163.90	137.76±20.86	86.50	226.20	153.68±46.50	0.449
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**							
Monosaccharaides (g)	9.00	25.90	17.16±6.22	7.90	38.50	21.03±9.73	0.407
Disaccharides (g)	16.30	61.90	36.63±17.23	19.30	113.00	42.48±28.63	0.663
Polysaccharides (g)	138.50	189.60	152.68±19.32	89.80	163.40	134.33±24.40	0.147

(*) Significance of the test at $p<0.05$, **S.D.**; Standard Deviation

Comparison of average intakes of monosaccharaides, disaccharides and polysaccharides revealed no significant differences between males and females within the three groups.

5.4.3.2 Assessment of fatty acids intake

Fatty acids are the major constituents of triglycerides and fall into the following categories: saturated fatty acids, *cis* monounsaturated fatty acids, *cis* polyunsaturated fatty acids (*n*–6 fatty acids and *n*–3 fatty acids), and *trans* fatty acids.

a. Saturated fatty acids (SFA)

The assessment of dietary saturated fatty acids consumed by all patients categories are shown in table 5.13.

Table 5.13 The daily intake of SFA in men and women in the three groups

SFA (g)	Overweight/Obese Patients Without Diabetes (n-47)			Overweight/Obese Diabetic Patients (n-167)			Normal Weight Diabetic Patients (n-71)			*p* value of ANOVA Kruskal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	*p* value for Student *t*-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	*p* value for Student *t*-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	*p* value for Student *t*-test	
Total SFA	32.38±15	34.0±8.6**	0.785	43.61±8.1	37.64±11	0.275	23.9±7**	23.7±8.9**	0.966	<0.001
Butyric acid C4,0	0.67±0.64	0.86±0.48	0.505	0.98±0.85	0.55±0.42	0.217	0.55±0.62	0.42±0.43	0.645	0.229
Caproic acid C6,0	0.44±0.41	0.55±0.28	0.519	0.63±0.53	0.36±0.27	0.223	0.36±0.38	0.25±0.27	0.525	0.171
Caprylic acid C8,0	0.65±0.59	0.57±0.35	0.740	0.41±0.29	0.82±0.74	0.233	0.21±0.21	0.27±0.34	0.705	0.010
Capric acid C10,0	0.84±0.68	0.86±0.40	0.934	0.93±0.62	1.02±0.67	0.801	0.48±0.46	0.4±0.3*	0.827	0.023
Lauric acid C12,0	1.56±1.21	1.40±0.76	0.731	1.60±1.15	3.12±2.93	0.253	0.75±0.68	0.7±0.6*	0.873	0.005
Myristic acid C14,0	3.28±2.25	3.71±1.28	0.632	4.03±2.27	3.48±1.89	0.623	2.28±1.61	1.88±1.2*	0.595	0.019
Pentad- acid C15,0	0.26±0.02	0.33±0.01	0.453	0.40±0.28	0.22±0.14	0.133	0.20±0.17	0.17±0.13	0.791	0.155
Palmitic acid C16,0	16.63±7.1*	17.5±4.5*	0.763	23.86±2.4	19.23±4.5	0.041	12.9±2.9*	13.66±5.8	0.781	0.001
Margaric acid C17,0	0.26±0.21	0.33±0.14	0.372	0.41±0.21	0.26±0.15	0.132	0.2±0.1**	0.18±0.10	0.864	0.055
Stearic acid C18,0	7.13±3.11	7.3±2.1**	0.853	9.71±1.07	7.85±1.95	0.055	5.46±1.23	5.3±1.99*	0.859	0.001
Arachidic acid C20,0	0.36±0.19	0.27±0.06*	0.211	0.35±0.10	0.40±0.13	0.452	0.2±0.08*	0.24±0.1*	0.435	0.007
Behenic acid C22,0	0.24±0.18	0.12±0.08	0.093	0.16±0.01	0.22±0.12	0.506	0.16±0.07	0.16±0.10	1.00	0.854

❶ We used the Student *t* test "independent samples" to compare means between men and women within each group of patients (Results are given as *p* value for Student *t*-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student *t* test "paired samples" (Results are indicated by asterisk *: $p<0.05$, **: $p<0.01$).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by *p* value for ANOVA Kruskal-Wallis test, in the last column).

SFA can be synthesized by the body, where they perform structural and metabolic functions. Neither an Estimated Average Requirement (EAR) and thus a Recommended Dietary Allowance (RDA) nor an Adequate Intake (AI) was set for saturated fatty acids because they are not essential and have no known role in preventing chronic disease.

It is recommended that individuals maintain their saturated fatty acid consumption as low as possible, while consuming a nutritionally adequate diet. Food sources of SFA tend to be animal-based foods, including whole milk, cream, butter, cheese, and fatty meats. Coconut oil, palm oil, and palm kernel oil are also high in saturated fatty acids (Otten *et al.*, 2006).

Comparing the dietary contribution of saturated fatty acids between males and females, no significant differences were found with exception for Palmitic acid (16:0) within overweight/obese diabetic patients.

Using ANOVA Kruskal-Wallis test to compare dietary intakes of SFA between the three groups of patients, we noticed a highly significant difference $p<0.001$ for total SFA.

High significant differences were further found in short- to medium-chain saturated fatty acids (8:0), (10:0), (12:0) and (14:0) with p values of about (0.010), (0.023), (0.005) and (0.019) respectively. However, long chain SFA that showed a significant differences between the three groups of patients included Palmitic acid ($p = 0.001$), Stearic acid ($p = 0.001$) and Arachidic acid ($p = 0.007$).

b. Monounsaturated fatty acids (MUFA)

Table 5.14 The daily intake of MUFA in men and women in the three groups

MUFA (g)	Overweight/Obese Patients Without Diabetes (n=47)			Overweight/Obese Diabetic Patients (n=167)			Normal Weight Diabetic Patients (n=71)			*p* value of ANOVA Krukal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	*p* value for Student *t*-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	*p* value for Student *t*-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	*p* value for Student *t*-test	
Total MUFA	31.24±11	29.6±8.2 **	0.742	40.13±10.3	30.64±10.7	0.113	23.2±8.1 **	29.17±18.4	0.475	0.082
Tetradece- acid C14,1	0.33±0.28	0.52±0.20	0.124	0.56±0.37	0.26±0.18	0.058	0.26±0.21	0.25±0.15	0.910	0.178
Palmitoleic acid C16,1	2.10±1.05 *	2.26±1.12 **	0.750	3.51±0.78	2.38±0.64	0.010	1.75±0.4 **	2.08±1.11	0.497	0.016
Oleic acid C18,1	27.55±9.7	26.01±7.3 *	0.709	36.35±13.3	26.44±10.4	0.130	20.55±7.8 *	26.25±17.3	0.467	0.144
Eicosenoic acid C20,1	0.57±0.55	0.33±0.08	0.211	0.60±0.41	0.71±0.53	0.674	0.33±0.10	0.28±0.16 *	0.577	0.028
Erucic acid C22,1	0.34±0.45	0.07±0.04	0.102	0.26±0.46	0.52±0.53	0.360	0.11±0.04	0.08±0.03	0.171	0.071

❶ We used the Student *t* test "independent samples" to compare means between men and women within each group of patients (Results are given as *p* value for Student *t*-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student *t* test "paired samples" (Results are indicated by asterisk *: $p<0.05$, **: $p<0.01$).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by *p* value for ANOVA Kruskal-Wallis test, in the last column).

Monounsaturated fatty acids (*n*-9) can be synthesized by the body and confer no known independent health benefits. Neither an EAR (and thus an RDA) nor an AI was set. Foods high in MUFA include canola oil, olive oil, high-oleic sunflower oil, high-oleic safflower oil, and animal products, primarily meat fat. Animal products provide about 50 % of dietary MUFA (Otten *et al.*, 2006).

One single significant difference was found between men and women in the group of overweight/obese diabetics for Palmitoleic acid 16:1 (p=0.010). Likewise, by comparing the three groups, significant differences were found for Palmitoleic acid 16:1 (p=0.016) and Eicosenoic acid 20:1 (p=0.028).

c. Polyunsaturated fatty acids (PUFA)

Table 5.15 The daily intake of PUFA in men and women within the three groups

PUFA (g)	Overweight/Obese Patients Without Diabetes (n=47)			Overweight/Obese Diabetic Patients (n=167)			Normal Weight Diabetic Patients (n=71)			*p* value of ANOVA Krukal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	*p* value for Student *t*-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	*p* value for Student *t*-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	*p* value for Student *t*-test	
Total PUFA	23.52±9.73	16.01±4.84	0.055	27.26±13.6	27.26±12.9	1.00	22.78±13.2	19.81±10.4	0.635	0.210
LA C 18,2	20.7±7.75	13.76±5.43	0.043	23.08±15.8	22.27±9.5	0.903	20.13±11.6	18.26±9.97	0.745	0.407
ALA C18,3	1.32±0.48	1.31±0.41	0.959	3.01±2.42	3.38±2.94	0.802	1.43±1.12	1.01±0.38 *	0.311	0.002
SDA C18,4	0.01±0.03	0.02±0.06	0.661	-	-	-	0.03±0.08	00.001	0.234	0.435
DGLA C20,3	0.26±0.36	0.07±0.04	0.138	0.20±0.34	0.38±0.38	0.354	0.08±0.04	0.04±0.05 *	0.152	0.030
AA C20,4 (g)	0.35±0.34	0.14±0.07	0.093	0.35±0.28	0.48±0.36	0.449	0.15±0.05	0.16±0.08 *	0.684	0.004
Adrenic acid C22,4	0.01±0.03	0.01±0.03	1.00	-	-	-	0.01±0.04	00.001	0.234	0.429
DPA C22,5	0.13±0.25	0.03±0.10	0.282	0.08±0.20	0.20±0.22	0.332	0.05±0.12	0.01±0.03	0.375	0.095
DHA C22,6	0.43±0.75	0.34±0.85	0.818	0.35±0.20	0.35±0.21	0.962	0.50±0.93	0.21±0.16	0.374	0.055

LA: Linoleic acid, **ALA**: α-linolenic acid, **SDA**: Stearidonic acid, **DGLA**: Dihomo-γ-linolenic acid, **AA**: Arachidonic acid, **DHA**: Docosahexaenoic acid, **DPA**: Docosapentaenoic acid

❶ We used the Student *t* test "independent samples" to compare means between men and women within each group of patients (Results are given as *p* value for Student *t*-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student *t* test "paired samples" (Results are indicated by asterisk *: $p<0.05$, **: $p<0.01$).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by *p* value for ANOVA Kruskal-Wallis test, in the last column).

PUFA are divided into *cis* polyunsaturated acids and *trans* polyunsaturated acids. *Cis* polyunsaturated acids include the *n*-6 fatty acids and *n*-3 fatty acids. Linoleic acid (18:2), an essential fatty acid (EFA) that cannot be made by the body, is the parent acid of the *n*-6 fatty acid series. Whilst, α-linolenic acid (ALA) (18:3) is the parent acid of the *n*-3 fatty acid series and must be obtained through the diet (Otten *et al.*, 2006).

A significant difference (p=0.043) in dietary intake of linoleic acid was found, using Student *t* test, between males and females in overweight/obese non-diabetic patients. However, the use of the non-parametric ANOVA Kruskal-Wallis test has highlighted

significant differences intake in ALA (p=0.002), dihomo-γ-linolenic acid (p=0.030) and Arachidonic acid (p=0.004).

5.4.3.3 Assessment of essential amino acids intake

Table 5.16 Essential amino acids obtained from the diet in each group

Amino acids (g)	Overweight/Obese Patients Without Diabetes (n=47)			Overweight/Obese Diabetic Patients (n=167)			Normal Weight Diabetic Patients (n=71)			Highest Median Intake (g/day)	p value of ANOVA Krukal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	p value for Student t-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	p value for Student t-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	p value for Student t-test		
His	2.57±0.75	2.32±0.91	0.527	2.71±0.64	2.02±0.66	0.066	1.91±0.89	1.86±0.40	0.884	**3.10**	0.069
Ile	4.85±1.10	4.22±1.64	0.351	4.85±1.28	3.88±1.18	0.160	3.66±1.58	3.67±0.74	0.986	**4.90**	0.145
Leu	7.57±1.69	6.61±2.52	0.354	7.50±1.86	6.04±1.81	0.156	5.76±2.51	5.61±1.12	0.872	**8.50**	0.092
Lys	6.58±2.09	5.88±2.91	0.567	7.01±1.68	5.18±1.83	0.073	5.05±2.83	4.80±1.09	0.812	**7.50**	0.114
Met	2.18±0.59	1.92±0.90	0.472	2.21±0.49	1.75±0.60	0.145	1.68±0.90	1.61±0.33	0.829	**2.50**	0.113
Phe	4.44±0.78	3.82±1.35	0.250	4.23±1.13	3.47±1.07	0.216	3.35±1.29	3.32±0.66	0.957	**4.80**	0.111
Thr	3.95±0.87	3.48±1.36	0.401	4.11±0.94	3.27±0.99	0.127	3.00±1.29	2.98±0.61	0.982	**4.20**	0.082
Trp	1.15±0.20	0.96±0.33	0.166	1.11±0.24	0.92±0.27	0.190	0.86±0.30	0.84±0.17	0.860	**1.30**	0.078
Val	5.22±1.09	4.62±1.88	0.420	5.41±1.36	4.36±1.39	0.175	3.95±1.61	3.91±0.76	0.951	**5.50**	0.070

His: Histidine, **Ile**: Isoleucine , **Leu**: Leucine , **Lys**: Lysine , **Met**: Methionine , **Phe**: Phenylalanine , **Thr**: Threonine , **Trp**: Tryptophane , **Val**: Valine

Highest Median Intake according to NRC, 2005

❶ We used the Student t test "independent samples" to compare means between men and women within each group of patients (Results are given as p value for Student t-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student t test "paired samples" (Results are indicated by asterisk *: p<0.05, **: p<0.01).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by p value for ANOVA Kruskal-Wallis test, in the last column).

The comparison between the two genders' results about the essential amino acids in each group did not show any significant difference. Likewise, the comparison between the three groups using the Kruskal-Wallis test showed no significant difference.

Proteins found in animal sources such as meat, poultry, fish, eggs, milk, cheese, and yogurt provide all nine indispensable amino acids and are referred to as "complete proteins". However, Proteins found in plants, legumes, grains, nuts, seeds, and vegetables tend to be

deficient in one or more of the indispensable amino acids and are called "incomplete proteins".

The comparison of indispensable amino acids dietary intakes, for the three groups, with the highest median intake references (NRC, 2005), showed no distinctive deficiencies in these proteins constituents.

5.4.4 Assessment of vitamin intake

Table 5.17 Vitamin status between men and women in the three groups

Vitamins	Overweight/Obese Patients Without Diabetes (n=47)			Overweight/Obese Diabetic Patients (n=167)			Normal Weight Diabetic Patients (n=71)			Requirements		*p* value of ANOVA Krukal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	*p* value for Student *t*-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	*p* value for Student *t*-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	*p* value for Student *t*-test	**Male**	**Female**	
Fat-Soluble Vitamins												
A (µg)	3792±6512	1377±496.8	0.284	1585±670	1535±472	0.867	1244±424	1703±925	0.280	**900**	**700**	0.729
D (µg)	4.51±8.09	5.12±9.97	0.888	2.75±2.63	2.73±3.05	0.991	5.81±11.86	1.67±1.46	0.311	**15**	**15**	0.764
E (mg)	23.65±6.45	14.78±6.52	0.010	19.18±17.4	18.28±6.25	0.888	20.08±13.2	19.53±9.85	0.928	**15**	**15**	0.764
K (µg)	592.5±234	474.1±212	0.278	470.13±219	528.85±186	0.586	539.96±121	530.83±310	0.947	120	90	0.986
Water-Soluble Vitamins												
C (mg)	194.75±76	108.41±42	0.009	141.40±43	151.77±82	0.783	137.31±44	122.55±32	0.468	**90**	**75**	0.790
B1 (mg)	1.40±0.39	1.10±0.30	0.088	1.31±0.35	1.23±0.28	0.621	1.10±0.32	1.02±0.27	0.629	**1.2**	**1.1**	0.112
B2 (mg)	1.88±1.26	1.18±0.38	0.133	1.38±0.29	1.31±0.32	0.669	1.26±0.17	1.32±0.24	0.645	**1.3**	**1.1**	0.933
B3 (mg)	26.56±9.49	21.08±9.70	0.244	19.31±3.67	16.61±4.69	0.257	20.71±13.3	18.67±4.19	0.672	**16**	**14**	0.095
B5 (mg)	6.90±2.86	4.37±1.60	0.035	5.25±1.58	4.92±1.31	0.669	4.63±0.88	4.86±1.04	0.661	5	5	0.454
B6 (mg)	2.61±0.57	1.83±0.82	0.034	2.28±0.22	2.11±0.61	0.525	2.28±0.95	1.73±0.36	0.135	**1.7**	**1.5**	0.212
B9 (µg)	393.43±18	229.95±100	0.031	266.53±116	260.34±79	0.904	304.7±60.6	264.27±66	0.253	**400**	**400**	0.660
B12 (µg)	6.28±0.97	4.28±4.47	0.585	6.05±3.14	4.30±2.94	0.293	1.00±0.92*	1.67±1.44*	0.330	**2.4**	**2.4**	0.006

Recommended Dietary Allowances (RDA) in **bold type**; Adequate Intakes (AI) underlined according to the Institute of Medicine, 2011.

❶ We used the Student *t* test "independent samples" to compare means between men and women within each group of patients (Results are given as *p* value for Student *t*-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student *t* test "paired samples" (Results are indicated by asterisk *: $p<0.05$, **: $p<0.01$).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by *p* value for ANOVA Kruskal-Wallis test, in the last column).

Through the analysis of the results, on dietary intake of vitamins in our patients (Table 5.17), many significant differences between the vitamin intake in men and women were observed in the overweight/obese non-diabetic group. These differences were observed on vitamin E (p=0.010), vitamin C (p=0.009) and vitamins of the B group, notably B5 (p=0.035), B6 (p=0.034) and B9 (p=0.031). One single difference between the three group was shown on vitamin B12 (p=0.006).

5.4.4.1 Fat-Soluble vitamins intake (A, D, E and K)

***Vitamin A**:* Preformed vitamin A (retinol) is found only in animal-based foods. However, foods that contain sufficient provitamin A carotenoids are deeply the coloured fruits and vegetables, or the fortified foods, such as margarine, some plant-based beverages, and cereals. In all our groups, the requirements for vitamin A are widely covered either in men or women.

***Vitamin D**:* The vitamin D has particular interest for its potential role in the pathogenesis and complications of diabetic disease and obesity. Vitamin D deficiency may impair the function of pancreatic cells and inhibit the secretion of insulin (Mosekilde, 2005). Contrariwise, a vitamin D supplementation would delay the conversion of glucose intolerance in diabetes (Schlienger *et al.*, 2010). Vitamin D may also affect diabetes control. Indeed, an inverse relationship between vitamin D levels and glycated hemoglobin was demonstrated.

Comparing to its RDA, we noticed that dietary intakes of this element were extremely low, in all patients groups, in women as in men.

***Vitamin E**:* The main dietary sources of vitamin E are vegetable oils, such as sunflower oil, canola oil, olive oil and palm oil. Other sources of vitamin E include unprocessed cereal grains, nuts, fruits, vegetables, and meats (especially the fatty portion). Food intakes of this element were sufficiently covered for all patients in our study ($\approx$15 mg /day).

***Vitamin K**:* Vitamin K functions as a coenzyme for biological reactions involved in blood coagulation and bone metabolism. Rich dietary sources of vitamin K include leafy green vegetables, soy and canola oils, and margarine. Vegetables particularly rich in vitamin K

include collard greens, spinach, and salad greens. Dietary intakes of vitamin K were covered by far in our population.

5.4.4.2 Water–Soluble vitamins intake

Vitamin C: Dietary intake of this vitamin is very adequate in the three groups of patients of both sexes. Foods rich in vitamin C include fruits and vegetables, including citrus fruits, tomatoes, potatoes, strawberries, spinach, and cruciferous vegetables.

Vitamin B_1: The group of normal weight diabetic patients does not sufficiently cover its needs in this vitamin. While the requirements of the two others groups were largely covered.

Food sources of thiamin include grain products, pork, ham, and fortified meat substitutes. The classic disease of thiamin deficiency is beriberi, which is sometimes seen in developing countries.

Vitamin B_2: Major food sources of riboflavin (vitamin B_2) include milk and milk drinks, bread products, and fortified cereals. Diseases such as cancer, cardiac disease, and diabetes mellitus are known to precipitate or exacerbate riboflavin deficiency.

We haven not found a B_2 deficiency in our groups except for men diabetic patients with normal weight.

Vitamin B_3: Meat, liver, poultry, and fish are rich sources of niacin. Other contributors to niacin intake include enriched and whole-grain breads and bread products and fortified ready-to-eat cereals. The needs for this vitamin were widely covered in all groups where they exceeded even the recommendations.

Vitamin B_5: Major food sources of pantothenic acid include chicken, beef, potatoes, oat cereals, tomato products, liver, kidney, yeast, egg yolk, broccoli, and whole grains.

Adequate intakes (AI) were not reached, although acceptable, in females of all groups as well as in normal weight diabetic males.

Vitamin B_6: Vitamin B_6 (pyridoxine) functions as a coenzyme in the metabolism of amino acids, glycogen, and sphingoid bases. Rich food sources of vitamin B_6 include highly fortified

cereals, beef liver and other organ meats, and highly fortified, soy-based meat substitutes. Dietary contributions of this vitamin were beyond the adequate intakes in all patients groups.

Vitamin B_9: Folate (B_9 vitamin) functions as a coenzyme in the metabolism of nucleic and amino acids. Rich food sources of folate include fortified grain products, dark green vegetables, and beans and legumes. We have found an inadequate intake of this vitamin in the entire studied cases.

Vitamin B_{12}: Vitamin B_{12} functions as a coenzyme for a critical reaction that converts homocysteine to methionine and in the metabolism of fatty acids of odd chain length. Vitamin B_{12} is naturally found in foods of animal origin. It is found in shellfish, organ meats such as liver, some game meats, and some fish (such as herring, sardines, and trout) but these are not a regular part of many people's diets. Deficiencies were noted among normal weight diabetic patients of both genders.

5.4.5 Assessment of minerals intake

Table 5.18 Mineral status between men and women in the three groups

Minerals	Overweight/Obese Patients Without Diabetes (n=47)			Overweight/Obese Diabetic Patients (n=167)			Normal Weight Diabetic Patients (n=71)			Requirements		*p* value of ANOVA Krukal-Wallis test
	Male 19 Means±S.D	**Female** 28 Means±S.D	*p* value for Student *t*-test	**Male** 49 Means±S.D	**Female** 118 Means±S.D	*p* value for Student *t*-test	**Male** 37 Means±S.D	**Female** 34 Means±S.D	*p* value for Student *t*-test	**Male**	**Female**	
Macro-elements												
Na (mg)	5622±825	4501±1876	0.120	7016±5045	4396±1493	0.161	5958±2669	4834±2283	0.398	1300	1300	0.813
K (mg)	3715±662	2708±715	0.007	3483±758	3281±690	0.603	3227±388	3034±381	0.358	4700	4700	0.668
Ca (mg)	777±282	488±152*	0.016	654.3±361	701.3±258	0.772	672.5±210	676.3±141	0.967	**1000**	**1200**	0.650
Mg (mg)	331±47.88	257±72.77	0.022	319±60.9	303±66.1	0.656	299.7±45	288±55.2	0.679	**420**	**320**	0.800
P (mg)	1489±264	1123±344	0.022	1375±387	1241±312	0.471	1168±277	1182±225	0.914	**700**	**700**	0.299
Cl (mg)	8866±1534	7094±2880	0.123	10744±7680	6859±2218	0.170	9370±3957	7604±3389	0.371	2000	2000	0.777
NaCl (g)	**13.61±2.3**	**11.12±4.6**	**0.167**	**17.0±12.6**	**10.64±3.5**	**0.169**	**14.68±6.5**	**11.86±5.6**	**0.390**	**< 5 g/day**	**< 5 g/day**	**0.780**
Trace-elements												
Fe (mg)	16.11±3.69	13.46±3.35	0.132	14.73±3.61	12.75±1.58	0.167	13.48±3.14	14.1±4.78	0.787	**8**	**8**	0.359
Zn (mg)	13.35±3.34	12.71±5.25	0.760	13.91±3.83	12.18±4.74	0.471	11.16±3.81	10.18±1.84	0.516	**11**	**8**	0.146
Cu (mg)	2.45±0.30	1.92±0.46	0.012	2.45±0.41	2.22±0.36	0.285	2.40±0.52	2.26±0.50	0.628	**0.9**	**0.9**	0.884
Mn (mg)	4.33±1.34*	2.67±1.27	0.016	2.78±0.65	2.82±0.68	0.915	3.33±0.76	3.67±2.26	0.728	2.3	1.8	0.143
I (µg)	91.6±19.1	72.76±25	0.092	125.4±86	102.9±82	0.618	97.6±33.4	84.8±16.3	0.338	**150**	**150**	0.696

Recommended Dietary Allowances (RDA) in **bold type**; Adequate Intakes (AI) underlined according to the Institut of Medecine 2011. NaCl requirements intake according to the Departement of Health and Human Services (DHHS, 2005) & WHO/FAO, 2003.

❶ We used the Student *t* test "independent samples" to compare means between men and women within each group of patients (Results are given as *p* value for Student *t*-test).
❷ For comparing results of men and women overweight/obese diabetics on the one hand, with patients of the same gender from the two other groups, on the other hand, we used the Student *t* test "paired samples" (Results are indicated by asterisk *: $p<0.05$, **: $p<0.01$).
❸ For comparing between the results of the three groups of patients, we used the Kruskal-Wallis test (Results are indicated by *p* value for ANOVA Kruskal-Wallis test, in the last column).

Table 5.18 displays mineral status in our three groups of patients. No difference between the two genders was observed with respect to macro- and micro-elements except for the non-diabetics overweight/obese individuals. In this last, we noticed a significant difference between men and women in potassium (p=0.007), calcium (p=0.016), magnesium (p=0.022) and phosphorus (p=0.022) as macro-elements and also in copper (p=0.012) and manganese (p=0.016) as micro-elements.

No differences were found between all groups of patients neither in macro-elements nor in micro-elements.

5.4.5.1 Macroelements

Sodium and chloride (Na and Cl): Sodium and chloride are necessary to maintain extracellular fluid volume and plasma osmolality. The sodium and the chloride are normally found in most foods together as sodium chloride (NaCl salt).

Dietary intakes of these minerals in all patients of the three groups were too high. Similarly, the sodium chloride intake (NaCl) was high and exceeded by far the requirements (5 g/day).

Potassium (K): The mineral potassium is the main intracellular cation in the body and is required for normal cellular function. Fruits and vegetables, particularly leafy greens, vine fruit, and root vegetables, are good food sources of potassium. Potassium intake among all patients remained low compared to the appropriate requirements.

Calcium (Ca): Foods rich in calcium include milk, yogurt, cheese, calcium-set tofu, calcium-fortified orange juice, Chinese cabbage, kale, and broccoli. Through our results, calcium requirements in the three patients groups were still far to be covered.

Magnesium (Mg): Foods rich in magnesium include green leafy vegetables, whole grains, and nuts. Meats, starches, and milk are intermediate in magnesium content, and refined foods generally have the lowest magnesium content. Epidemiological and multicentric studies have registered an inverse relationship between the ingestion of food rich in Mg and the risk of diabetes (Nadler *et al.*, 2004). We noted that dietary intakes of magnesium remained lower than requirements in all patients of both genders.

Phosphorus (P): Phosphorus is the most abundant mineral in the body next to calcium. This mineral is needed for the growth, maintenance, and repair of all tissues and cells, and for the production of the genetic building blocks, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Phosphorus is also needed to help balance and use other vitamins and minerals, including vitamin D, iodine, magnesium, and zinc (DHHS, 2005).

The mineral is found in milk, grains, and protein rich foods. Some health conditions such as diabetes, starvation, and alcoholism can cause levels of phosphorus in the body to fall.

Most of patients with both genders in the three groups get sufficiently phosphorus in their diets.

5.4.5.2 Trace element

Iron (Fe): About half of dietary intake of iron is obtained from meat, poultry, and fish as heme iron, which is highly bioavailable; the remainder is non-heme, which is less readily absorbed by the body. Iron in dairy foods, eggs, and all plant-based foods is entirely non-heme. Iron overload is a risk factor for diabetes. The link between iron and diabetes was first recognized in pathologic conditions hereditary hemochromatosis and thalassemia but high levels of dietary iron also impart diabetes risk (Otten *et al.*, 2006; Simcox & McClain, 2013).

Iron intake in patients of the three groups exceeded recommended guidelines set at 8 mg/day by Otten *et al.*, 2006.

Zinc (Zn): Foods rich in zinc include meat, some shellfish, legumes, fortified cereals, and whole grains. Zn plays a key role in the storage and secretion of insulin, which subsequently increases the uptake of glucose (Kazi *et al.*, 2008). The decreased plasma level of Zn adversely affects the ability of islet cells to produce and secrete insulin (Rungby, 2010; Brender *et al.*, 2010). All patients of the three groups had an adequate zinc intake.

Copper (Cu): Sources of copper include organ meats, seafood, nuts, seeds, wheat-bran cereals, and whole-grain products.

Recently, it has been reported that disturbances in copper levels in various bio-fluids and tissues are associated with abnormalities implicated in metabolic pathways of diabetes and its complications (Kazi *et al.*, 2008).We noticed that copper requirements in all patients were quite sufficient.

Manganese (Mn): The highest contributors of manganese to the diet are grains, beverages (tea), and vegetables. Mn is also required for normal insulin synthesis, its secretion, and an alteration in its metabolism has been implicated in diabetes development (Kazi *et al.*, 2008).

Manganese dietary intakes were sufficiently filled and exceeded adequate intakes in the three groups.

Iodine (I): The primary source of iodine is the diet via consumption of foods that have been fortified with iodine, including salt, dairy products and bread, or that are naturally abundant in the micronutrient, such as seafood (Leung & Braverman, 2014). According to our results (Table 5.18), Iodine intake may be considered sufficient in overweight/obese diabetic patients of both genders and in normal weight diabetic males. However, iodine intakes remained still inadequate in the other patients.

5.5 Blood Parameters

5.5.1 Assessment of fasting blood parameters

Biochemical blood parameters concerning glucose, total cholesterol, HDL-c, LDL-c and TG were evaluated during fasting and postprandial periods, both in males and females. Results are shown in table 5.19 and table 5.20

Table 5.19 Comparison of fasting biochemical blood parameters between males and females within each group

Fasting Blood Parameters	Male			Female			Requirements		*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	Male	Female	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**									
Glucose (g/L)	0.95	1.15	1.04±0.06	0.89	1.30	1.07±0.09	1.10	1.10	0.213
Total Cholesterol (g/L)	1.14	2.23	1.56±0.35	0.92	2.22	1.36±0.31	<2.40	<2.40	0.052
HDL-Cholesterol (g/L)	0.28	0.41	0.35±0.03	0.24	0.45	0.35±0.04	>0.40	>0.50	0.635
LDL-Cholesterol (g/L)	0.90	1.29	1.15±0.10	0.37	1.68	1.15±0.29	<1.30	<1.30	0.987
Triglycerides (g/L)	0.59	2.23	1.69±0.44	0.79	2.25	1.69±0.45	<1.50	<1.50	0.977
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**									
Glucose (g/L)	0.76	3.14	1.70±0.55	0.35	3.16	1.55±0.64	1.30	1.30	0.144
Total Cholesterol (g/L)	1.12	2.60	1.71±0.37	0.87	2.71	1.69±0.36	<2.00	<2.00	0.738
HDL-Cholesterol (g/L)	0.18	0.87	0.38±0.13	0.19	0.91	0.40±0.10	>0.40	>0.50	0.412
LDL-Cholesterol (g/L)	0.58	2.00	1.06±0.32	0.26	2.16	1.04±0.32	<1.00	<1.00	0.744
Triglycerides (g/L)	0.49	3.14	1.30±0.59	0.42	5.10	1.52±0.74	<1.50	<1.50	0.073
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**									
Glucose (g/L)	0.74	3.87	1.82±0.71	0.84	2.38	1.38±0.36	1.30	1.30	0.002
Total Cholesterol (g/L)	0.73	2.29	1.66±0.35	1.12	2.45	1.72±0.31	<2.00	<2.00	0.476
HDL-Cholesterol (g/L)	0.12	0.67	0.35±0.11	0.20	0.74	0.39±0.13	>0.40	>0.50	0.265
LDL-Cholesterol (g/L)	0.25	1.88	1.08±0.38	0.48	1.68	1.16±0.29	<1.00	<1.00	0.311
Triglycerides (g/L)	0.09	3.53	1.26±0.65	0.41	4.16	1.44±0.82	<1.50	<1.50	0.325

(*)Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **HDL**; High Density Lipoprotein, **LDL**; Low Density Lipoprotein, **S.D.**: Standard Deviation

Lipid requirements according to the American Association of Clinical Endocrinologists AACE (Jellinger *et al.*, 2012) & glucose requirements according to the American Diabetes Association (ADA, 2014).

5.5.2 Assessment of postprandial blood parameters

Table 5.20 Comparison of postprandial biochemical blood parameters between males and females within each group

Postprandial Blood Parameters	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**							
Glucose (g/L)	1.20	2.03	1.66±0.28	1.23	2.10	1.68±0.26	0.818
Total Cholesterol (g/L)	1.04	2.21	1.61±0.31	1.04	2.85	1.68±0.35	0.489
HDL-Cholesterol (g/L)	0.25	0.41	0.35±0.04	0.29	0.70	0.40±0.11	0.048
LDL-Cholesterol (g/L)	0.98	1.85	1.33±0.30	0.55	1.98	1.32±0.34	0.927
Triglycerides (g/L)	1.04	2.56	1.94±0.48	0.70	3.01	1.93±0.54	0.986
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**							
Glucose (g/L)	0.82	6.45	2.38±1.15	0.35	5.08	2.23±0.96	0.383
Total Cholesterol (g/L)	0.85	3.78	1.76±0.48	0.85	2.90	1.82±0.45	0.505
HDL-Cholesterol (g/L)	0.17	0.76	0.35±0.12	0.16	0.76	0.39±0.11	0.029
LDL-Cholesterol (g/L)	0.47	3.06	1.12±0.42	0.31	1.99	1.08±0.36	0.529
Triglycerides (g/L)	0.40	6.39	1.60±1.00	0.35	4.47	1.70±0.80	0.478
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**							
Glucose (g/L)	1.28	5.08	2.48±1.17	0.96	5.08	2.30±0.95	0.473
Total Cholesterol (g/L)	0.75	2.47	1.68±0.43	1.07	2.36	1.78±0.36	0.304
HDL-Cholesterol (g/L)	0.11	0.70	0.34±0.12	0.16	0.53	0.36±0.10	0.388
LDL-Cholesterol (g/L)	0.25	1.70	1.07±0.36	0.60	1.73	1.10±0.28	0.722
Triglycerides (g/L)	0.65	6.39	1.49±0.99	0.40	3.58	1.58±0.89	0.670

*Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **HDL**; High Density Lipoprotein, **LDL**; Low Density Lipoprotein, **S.D.**: Standard Deviation

The comparison of fasting biochemical parameters (lipid and glucose profiles) between the two genders (Table 5.19), within each group of patients, showed no significant differences with an exception regarding the fasting glycaemia in normal weight diabetic patients group (p=0.002).

Based on the AACE guidelines (Jellinger *et al.*, 2012), a moderate hypertriglyceridemia was identified in overweight/obese non-diabetic subjects, of both

genders, and in overweight/obese diabetic women. However, HDL-c levels were low in all patients, and LDL-c levels were higher in the two groups of diabetic patients.

During the postprandial state (Table 5.20), significant differences were noted for HDL-c levels between men and women in overweight/obese patients, whether diabetics (p=0.048) or non-diabetics (p=0.029). However, regarding the glucose homeostasis, we found a high postprandial glycaemia, compared to the recommendations of the ADA (2014), in all patients of the three groups.

5.5.3 Assessment of non-fasting blood parameters

Table 5.21 Comparison of non-fasting apolipoproteins and HbA1c between males and females within each group

Non-fasting Blood Parameters	Male			Female			Requirements		p value for Student t-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	Male	Female	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**									
HbA1c (%)	5.10	6.89	6.43±0.49	5.14	7.00	6.31±0.55	< 6.0	< 6.0	0.462
apo A1 (g/L)	1.11	1.78	1.35±0.18	0.89	1.78	1.23±0.21	≈ 1.0	≈ 1.0	0.060
apo B (g/L)	0.54	1.04	0.86±0.14	0.49	1.16	0.85±0.16	< 0.8	< 0.8	0.832
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**									
HbA1c (%)	5.10	10.14	7.54±1.17	5.00	10.98	7.57±1.25	< 7.0	< 7.0	0.853
apo A1 (g/L)	0.66	2.44	1.24±0.43	0.62	2.44	1.35±0.38	≈ 1.0	≈ 1.0	0.104
apo B (g/L)	0.39	1.96	0.87±0.32	0.36	3.33	1.01±0.49	< 0.9	< 0.9	0.073
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**									
HbA1c (%)	5.44	11.00	7.55±1.25	5.80	11.19	7.79±1.29	< 7.0	< 7.0	0.413
apo A1 (g/L)	0.73	2.74	1.15±0.44	0.62	1.78	1.26±0.27	≈ 1.0	≈ 1.0	0.199
apo B (g/L)	0.42	1.48	0.89±0.23	0.36	2.00	0.92±0.32	< 0.9	< 0.9	0.661

(*)Significantly different at p<0.05, **Min.**; minimum, **Max.**; Maximum, **HbA1c**; Glycated Hemoglobin (A1C), **apo A1**; apolipoprotein A1, **apo B**: apolipoprotein B.

HbA1c requirements according to the American Diabetes Association (ADA, 2014). Apolipoproteins requirements according to AACE guidelines (Jellinger *et al.*, 2012).

ApoB and apoA 1 are the two major apolipoproteins involved in lipid transport in the body. ApoB is the major protein in Very Low Density (VLDL), Intermediate Density (IDL)

and LDL. apoA 1 is the major protein in HDL particles. The comparison of blood levels of these apolipoproteins did not indicate any significant differences between men and women in the three groups of patients, although their rates were higher than target recommendations of the AACE (Jellinger *et al.*, 2012).

On the other hand, levels of HbA1c (haemoglobin which is measured primarily to identify the average plasma glucose concentration over prolonged periods of time) were slightly higher than ADA (2014) requirements for all patients, but no significant differences were observed between the two genders (Table 5.21).

5.5.4 Comparison of blood parameters between the groups

Table 5.22 Comparison of glucose and lipids levels during fasting, postprandial and non-fasting states between the three groups of patients

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal-Wallis test Chi-squared value (X^2)	*p* value of asymptotic significance*
Fasting State							
Glucose (g/L)	1.04 1.06±0.08	*p*<0.001	1.45 1.59±0.62	*p*=0.900	1.46 1.60±0.61	42.288	<0.001
Total Cholesterol (g/L)	1.36 1.44±0.34	*p*<0.001	1.74 1.70±0.36	*p*=0.765	1.66 1.69±0.33	20.110	<0.001
HDL-Cholesterol (g/L)	0.36 0.35±0.04	*p*=0.125	0.37 0.39±0.11	*p*=0.283	0.37 0.37±0.12	2.950	0.229
LDL-Cholesterol (g/L)	1.18 1.15±0.23	*p*=0.003	1.06 1.05±0.32	*p*=0.053	1.17 1.12±0.34	9.961	0.007
Triglycerides (g/L)	1.84 1.69±0.44	*p*=0.001	1.31 1.46±0.70	*p*=0.104	1.11 1.35±0.74	17.149	<0.001
Postprandial State							
Glucose (g/L)	1.77 1.68±0.26	*p*<0.001	2.05 2.27±1.02	*p*=0.509	2.02 2.39±1.07	20.248	<0.001
Total Cholesterol (g/L)	1.65 1.65±0.33	*p*=0.025	1.80 1.80±0.46	*p*=0.266	1.75 1.73±0.40	5.352	0.069
HDL-Cholesterol (g/L)	0.38 0.38±0.09	*p*=0.841	0.38 0.38±0.12	*p*=0.057	0.34 0.35±0.11	4.417	0.110
LDL-Cholesterol (g/L)	1.25 1.33±0.33	*p*<0.001	1.10 1.09±0.37	*p*=0.974	1.09 1.08±0.32	18.086	<0.001
Triglycerides (g/L)	2.12 1.93±0.51	*p*=0.001	1.52 1.67±0.86	*p*=0.064	1.27 1.53±0.94	18.222	<0.001
Non-Fasting State							
HbA1c (%)	6.50 6.36±0.52	*p*<0.001	7.40 7.56±1.22	*p*=0.888	7.40 7.67±1.26	57.250	<0.001
apo A1 (g/L)	1.19 1.28±0.20	*p*=0.906	1.28 1.32±0.40	*p*=0.027	1.17 1.21±0.37	5.591	0.061
apo B (g/L)	0.87 0.85±0.15	*p*=0.288	0.91 0.97±0.45	*p*=0.570	0.87 0.90±0.28	1.229	0.541

(*)Significance of the test at $p<0.05$, **HDL**; High Density Lipoprotein, **LDL**; Low Density Lipoprotein; **HbA1c**; Glycated Hemoglobin (A1C); **apo A1**; apolipoprotein A1, **apo B**: apolipoprotein B.

The evaluation during the fasting state indicated a very high significant differences of blood glucose, total cholesterol, LDL-c and triglycerides between the three groups (using the

Kruskal-Wallis test), and also between the two groups of overweight/obese patients (non-diabetic patients and T2D ones).

During the postprandial state, significant differences were noted for glucose, LDL- c and TG between the three groups, in addition to total cholesterol, when we consider the two groups of overweight/obese patients. On the other hand, for the non-fasting parameters, high significant differences were noted for HbA1c between the three groups and between the two groups of overweight/obese patients. Similarly, for apo A1 between the two groups of T2D patients (with overweight/obesity or normal weight).

5.6 Evaluation of Blood Lipid Ratios

5.6.1 Assessment of fasting lipid ratios

Table 5.23 Comparison of fasting biochemical blood ratios between males and females within each group

Fasting Lipid Ratios	Male			Female			Requirements		*p* value for Student *t*-test *
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	Male	Female	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**									
Total cholesterol / HDL-c	2.92	6.19	4.46±0.97	2.30	6.06	3.87±0.96	< 4.0	< 3.5	0.049
LDL-c / HDL-c	2.24	4.39	3.32±0.49	0.92	5.17	3.29±0.93	< 2.5	< 2.5	0.895
Triglycerides / HDL -c	1.44	7.07	4.89±1.44	1.98	8.75	4.85±1.66	< 3.0	< 3.0	0.945
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**									
Total cholesterol / HDL-c	1.67	8.28	4.79±1.53	1.87	7.48	4.42±1.27	< 4.0	< 3.5	0.113
LDL-c / HDL-c	0.79	6.38	3.02±1.29	0.63	5.84	2.75±1.06	< 2.5	< 2.5	0.159
Triglycerides / HDL -c	0.92	8.33	3.70±1.79	0.79	13.78	4.05±2.28	< 3.0	< 3.0	0.337
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**									
Total cholesterol / HDL-c	2.04	7.05	4.95±1.31	2.05	9.45	4.89±2.00	< 4.0	< 3.5	0.878
LDL-c / HDL-c	0.81	5.33	3.19±1.25	1.20	7.35	3.35±1.65	< 2.5	< 2.5	0.643
Triglycerides / HDL -c	0.25	14.50	3.95±2.61	1.19	11.56	4.22±2.88	< 3.0	< 3.0	0.688

(*)Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **HDL-c**; High Density Lipoprotein cholesterol, **LDL-c**; Low Density Lipoprotein cholesterol, **S.D.**: Standard Deviation

Requirements according to Pereira (2012).

The use of lipid ratios, as the LDL-c/HDL-c ratio and the total cholesterol/HDL-c ratio is an alternative option, with very promising results, in the context of cardiovascular risk

stratification and assessment of the effectiveness of lipid-lowering interventions. Changes in these relations have in fact been shown to better indicate the reduction in cardiovascular risk compared with the absolute levels of conventionally used lipid measures (Natarajan *et al.*, 2003; Kannel, 2005).

Table 5.23 shows the comparison of fasting biochemical blood ratios between males and females within the three groups. With the exception of total cholesterol/HDL-c in overweight/obese without T2D (p=0.049), no significant differences were found comparing the lipid ratios between men and women within the three groups.

By comparing our results with the optimal levels of recommendations established by Pereira (2012), we noticed that all lipid ratios of our patients (both genders) were beyond the requirements. These results may suggest a high cardiovascular risk in our population.

5.6.2 Assessment of postprandial lipid ratios

Table 5.24 Comparison of postprandial biochemical blood ratios between males and females within each group

Postprandial lipid ratios	Male			Female			*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**							
Total cholesterol / HDL-c	2.97	7.13	4.62±1.09	2.58	7.31	4.29±1.13	0.324
LDL-c / HDL-c	2.44	7.40	3.86±1.15	1.40	5.66	3.36±0.98	0.119
Triglycerides / HDL -c	2.67	8.60	5.59±1.65	1.00	7.72	4.93±1.47	0.163
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**							
Total cholesterol / HDL-c	2.49	15.12	5.40±2.06	2.49	10.20	4.86±1.63	0.074
LDL-c / HDL-c	1.24	12.24	3.48±1.79	1.19	7.95	2.92±1.29	0.027
Triglycerides / HDL -c	0.88	18.26	5.00±3.17	0.66	17.90	4.68±2.66	0.503
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**							
Total cholesterol / HDL-c	2.80	8.58	5.24±1.30	2.94	10.20	5.31±1.88	0.867
LDL-c / HDL-c	1.25	5.27	3.32±1.12	1.40	5.60	3.29±1.26	0.907
Triglycerides / HDL -c	1.11	18.26	4.94±3.63	1.10	17.90	5.08±4.12	0.878

(*)Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **HDL-c**; High Density Lipoprotein cholesterol, **LDL-c**; Low Density Lipoprotein cholesterol, **S.D.**: Standard Deviation

In the absence of coherent references that can serve as recommendations for blood lipid ratios during the postprandial state, our results (Table 5.24) did not show significant differences in postprandial lipid ratios between males and females except for LDL-c/HDL-c ratio in overweight/obese patients with T2D.

5.6.3 Assessment of apolipoproteins ratios

Table 5.25 Comparison of apolipoproteins ratios between males and females within each group

Apolipoproteins Ratios	Male			Female			Requirements		*p* value for Student *t*-test*
	Min.	Max.	Means±S.D	Min.	Max.	Means±S.D	Male	Female	
Overweight/Obese Patients Without Diabetes (n=47, 19M and 28F**)**									
apo B / apo A1	0.34	0.82	0.65±0.14	0.33	1.25	0.70±0.17	0.7-0.89	0.6-0.79	0.278
apo A1 / F HDL-c	2.95	6.36	3.89±0.85	2.34	4.81	3.51±0.71	> 3.43	> 3.43	0.096
apo A1 / PP HDL-c	3.03	5.93	3.88±0.77	1.67	5.07	3.15±0.69	–	–	0.002
Overweight/Obese Diabetic Patients (n=167, 49M and 118 F**)**									
apo B / apo A1	0.26	2.45	0.76±0.36	0.26	2.27	0.78±0.36	0.7-0.89	0.6-0.79	0.773
apo A1 / F HDL-c	1.31	6.43	3.37±1.05	1.60	9.04	3.52±1.20	> 3.43	> 3.43	0.440
apo A1 / PP HDL-c	2.28	6.14	3.58±0.71	2.11	5.85	3.46±0.59	–	–	0.253
Normal Weight Diabetic Patients (n=71, 37M and 34F**)**									
apo B / apo A1	0.40	1.44	0.81±0.24	0.32	1.77	0.75±0.29	0.7-0.89	0.6-0.79	0.335
apo A1 / F HDL-c	1.42	6.08	3.44±1.23	1.19	6.27	3.59±1.31	> 3.43	> 3.43	0.618
apo A1 / PP HDL-c	2.44	6.64	3.49±0.79	2.11	5.85	3.64±0.86	–	–	0.450

(*)Significantly different at $p<0.05$, **Min.**; minimum, **Max.**; Maximum, **F HDL-c**; Fasting High Density Lipoprotein cholesterol, **PP HDL-c;** Postprandial High Density Lipoprotein cholesterol, **S.D.**: Standard Deviation; **apo A1**; apolipoprotein A1, **apo B**: apolipoprotein B.
Apo B/apo A1 requirements according to Walldius *et al.*, 2001 and Yusuf *et al.*, 2004. Apo A1/HDL-c according to the National Cholesterol Education Program (NCEP, 2001).

An accumulating body of data indicates that the apo B/apo A1 ratio is a powerful marker of risk for future cardiovascular disease (Sierra-Johnson *et al.*, 2009).The comparison of apo B/apo A1, apo A1/F HDL-c and apo A1/PP HDL-c ratios between males and females by using student *t*-test, indicated no significant differences with an exception for apo A1/PP HDL-c ratio ($p = 0.002$) in the overweight/obese patients without T2D.

Values of apo B/apo A-I ratio during therapy should preferably be reduced to <0.7 or even to lower levels in patients with great risk (Nissen *et al.*, 2006). After adapting our data with the risk thresholds of AMORIS (Walldius *et al.*, 2001) and INTERHEART (Yusuf *et*

al., 2004) studies, men and women in the three groups, are prone to moderate (from 0.7 to 0.89 in men and 0.60 to 0.79 in women) cardiovascular risks (myocardial infarction). However, apo A1/F HDL-c ratios don't show a real risk comparing to NCEP (2001) ranges (Table 5.25).

5.6.4 Comparison of lipid ratios between the groups

Table 5.26 Variations of non-fasting apolipoproteins ratio, fasting and postprandial lipid ratios between the three groups of patients

	Overweight/Obese Patients Without Diabetes (n=47) Median Means±S.D		Overweight/Obese Diabetic Patients (n=167) Median Means±S.D		Normal Weight Diabetic Patients (n=71) Median Means±S.D	ANOVA Kruskal-Wallis test Chi-squared value (X^2)	ANOVA Kruskal-Wallis test p value of asymptotic significance*
Fasting State							
Total cholesterol / HDL-c	3.97 4.11±1.00	p=0.101	4.34 4.53±1.36	p=0.134	4.56 4.92±1.66	7.06	0.029
LDL-c / HDL-c	3.27 3.30±0.77	p<0.001	2.68 2.83±1.13	p=0.046	3.12 3.26±1.45	15.46	<0.001
Triglycerides / HDL -c	4.89 4.87±1.56	p=0.101	3.39 3.95±2.15	p=0.706	3.18 4.08±2.73	14.79	0.001
Postprandial State							
Total cholesterol / HDL-c	4.45 4.42±1.11	P=0.058	4.71 5.02±1.78	p=0.147	5.00 5.27±1.60	7.80	0.020
LDL-c / HDL-c	3.47 3.57±1.07	p=0.006	2.90 3.09±1.47	p=0.088	3.13 3.30±1.18	8.89	0.012
Triglycerides / HDL -c	5.43 5.20±1.56	P=0.012	4.15 4.77±2.81	p=0.617	4.03 5.01±3.84	8.08	0.018
Non Fasting State							
apo B / apo A1	0.67 0.68±0.16	P=0.331	0.70 0.77±0.36	p=0.322	0.78 0.78±0.27	3.48	0.175
apo A1 / F HDL-c	3.52 3.66±0.78	p=0.145	3.47 3.48±1.16	p=0.716	3.42 3.51±1.26	1.85	0.396
apo A1 / PP HDL-C	3.36 3.44±0.80	p=0.525	3.47 3.49±0.63	p=0.842	3.47 3.56±0.79	0.55	0.757

(*)Significance of the test at p<0.05; **HDL-c**; High Density Lipoprotein cholesterol, **LDL-c**; Low Density Lipoprotein cholesterol; **F HDL-c**; Fasting High Density Lipoprotein cholesterol, **PP HDL-c**; Postprandial High Density Lipoprotein cholesterol; **apo A1**; apolipoprotein A1, **apo B**: apolipoprotein B.

During both fasting and postprandial states, there were high significant differences of total cholesterol/HDL-c, LDL-c/HDL-c and TG/HDL-c ratios between the three groups of patients and between the two groups of overweight/obese patients. Nevertheless, no significant differences were noticed, neither between the three groups nor between each two groups as possible pairwise combinations (Table 5.26).

5.6.5 Correlation of apo B/apo A1 ratio with conventional lipid ratios

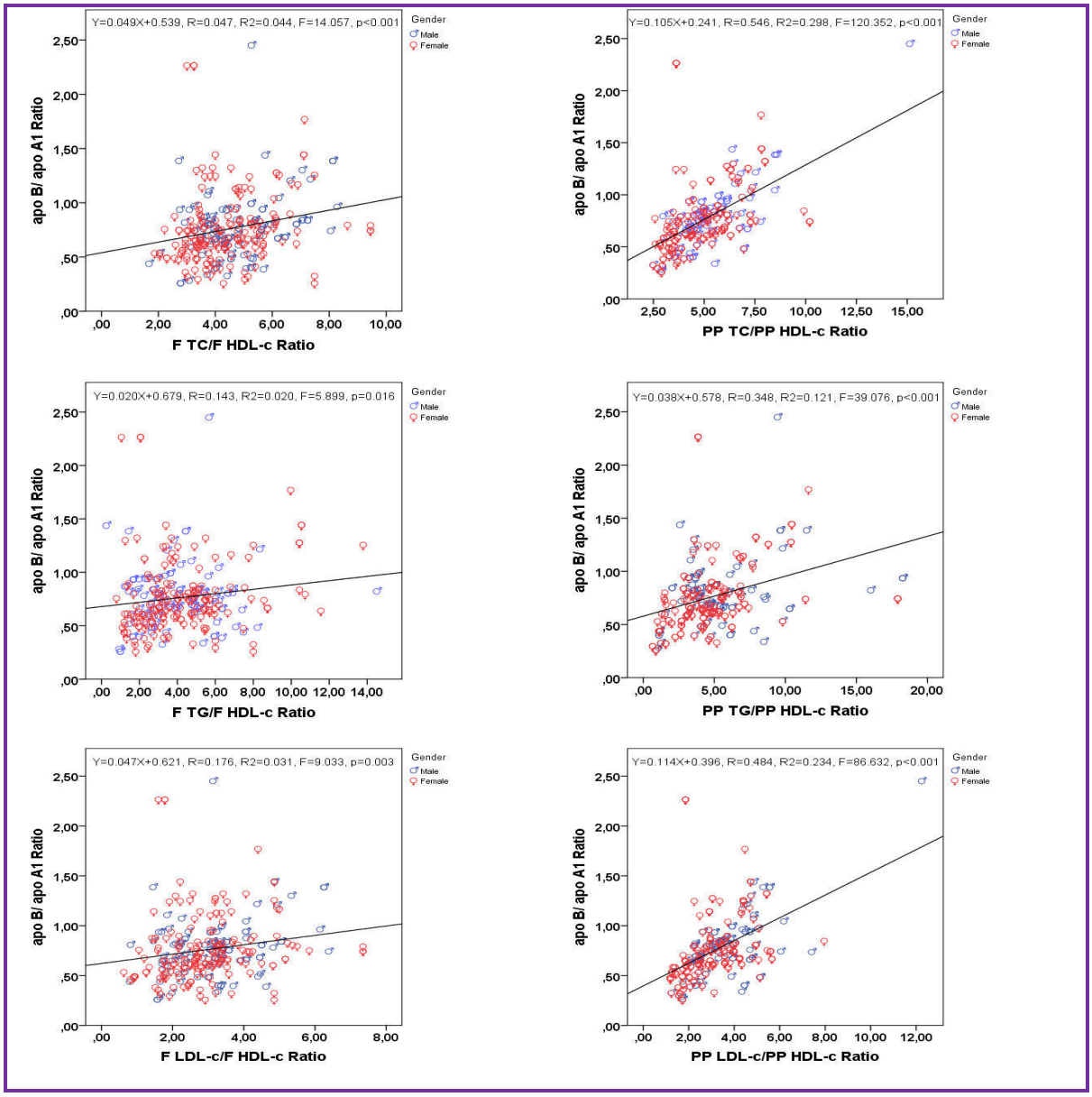


Figure 5.7 Mutual association between apo B/apo A1 ratio and conventional lipid ratios during fasting and postprandial states.

As illustrated in figure 5.6, the apo B/apo A-I ratio was significantly correlated with TC/HDL-c, TG/HDL-c and LDL-c/HDL-c ratios both in fasting and in postprandial states. However, we noticed that the apo B/apo A-I ratio provides the best growing accurate trend when the postprandial TC/HDL-c (p<0.001, r^2=0.298, F=120.352) and LDL-c/HDL-c (p<0.001, r^2=0.234, F=86.632) ratios increase.

General Discussion

General Discussion

Diabetes mellitus is one of the most prevalent chronic diseases in almost all countries. Between 2010 and 2030, the number of adults with diagnosed diabetes is expectedto increase by 69% in developing countries and by 20% in developed countries (Shaw *et al.*, 2010). Concurrently, the prevalence of obesity is increasing dramaticallyall over the world (James, 2008).

Measured by BMI, obesity is regarded as a powerful environmental risk factor for T2D and its co-morbidities and has increased clearly during the last decades. Estimates suggest that obesity will affect up to 1 billion people in 2030 (Kelly *et al.*, 2008).

Assessment of health risks, including diabetes and obesity, are measured in clinical settings and epidemiological research by referring usually to fasting recommendations. These latter are originally introduced to decrease variability and achieve consistency in the metabolic states of patients at the time of sample collection. Several recent studies, however, suggest that the measurement of metabolism indicators during the postprandial state is an acceptablealternative. Recently there has been a rising interest in understanding postprandial glucose and lipoprotein metabolism. This interest reflects early predictions that impaired postprandial metabolism may increase the atherogenic potential. Postprandial lipemia is a complex phenomenon that follows the intake of fat-rich meal. Hence, postprandial hyperlipidemia, which characterizes such states as obesity, the metabolic syndrome or T2D, is assumed to favour arteriosclerosis. Both postprandial hyperglycemia and hyperlipemia have been identified as risk markers for cardiovascular disease. Our investigation aimed to address this issue by systematically comparing both fasting and postprandial metabolic responses elicited by carbohydrate, fat and protein in obese patients with T2D and to compare these

responses with two other groups as control; obese individuals without diabetes and non-obese ones with T2D.

Our study was conducted on 285 subjects divided into three groups (47 non-diabetic individuals with BMI ≥ 25, 167 diabetic patients with BMI ≥ 25 and 71 normal weight diabetics). First, we compared the anthropometric parameters between men and women within each group of patients. Our finding revealed that men had often higher weight and waist circumference. However, women had higher BMI values. These results are consistent with data of many studies (Parikh*et al.*, 2002; Amrane&Khaled, 2012; Cox-York *et al.*, 2013; Fogal*et al.*, 2014).

Overweight, obesity and diabetes are co-morbid diseases that frequently confound hypertension, which add significantly to their overall morbidity and mortality (Sowers *et al.*, 2001). Our results suggest that blood pressure averages were at the limit of the recommended values; nevertheless, the maximum values of systolic and diastolic pressure exceeded by far the recommended levels (140/80-85-90 mmHg) for many patients according to their groups. Likewise, systolic pressure in overweight/obese diabetic patients was higher than systolic pressure in the two other groups. The same findings were reported by Fukuda *et al.*, 2015.

Comparing between men and women, systolic blood pressure was moderately high in females. The increase in systolic blood pressure is partly explained by a decrease in arterial compliance with age which is significantly correlated with modest weight gain and the impact of insulin resistance (von Bibra*et al.*, 2014). After 50 years of age, hypertension is more common in menopausal women.

Physiologically, the accumulation and the enlargement of adipose tissue is associated with the increase in the number of adipose tissue macrophages, which are responsible for the increased plasma concentration of pro-inflammatory cytokines and more precisely IL-6 and

TNF-α expression (Weisberg *et al.*, 2003). Recent works of Lukic*et al.* (2014) have confirmed that the increased levels of these two cytokines (IL-6 and TNF-α), in patients with T2D who suffer from an excess weight (overweight or obese), lead to an increase in systolic and diastolic blood pressure.

Through the analysis of questionnaires, and in terms of marital status, we noticed that the majority of patients in the three groups were married (> 60%). It should be emphasized too that 6.38% of overweight/obese non-diabetic patients, 16.77% of overweight/obese diabetic patients and 8.45% of normal weight diabetic patients, were widow (er). These findings are similar to those obtained by Otero *et al.* (2007).

According to Rodriguez & Guerrero (1997), psychosocial variables have an important effect on patient's disease that interferes in family dynamics. An unfavourable family environment can interfere in patient compliance with treatment. Organized and structured families provide a more appropriate environment to support diabetic or obese patient health care. In the same context, losing one's spouse causes health changes, such as depression, dismay and loss of the will to live.

The family concept is very important and may influence the behaviour of diabetic patients towards their disease and make them collaborate to obtain good metabolic control.

In the present study, we reported an association between illiteracy and T2D in the two groups of diabetic patients; more than 35% of diabetic patients had no educational level, which is consistent with other studies (Gupta *et al.*, 2003; Al-Moosa*et al.*, 2006; Laramee*et al.*, 2007; Veghari*et al.*, 2010). Moreover, individuals with limited literacy have no access to knowledge about medical conditions and about self care for the prevention or treatment of T2D and other diseases (Schillinger*et al.*, 2002; Scott *et al.*, 2002).

Regarding occupation, more participants had no professional activities and 41.86% were still working. These data agree with our subjects' age. Figuring out the mechanisms at work is very important for understanding behavioural changes, metabolic syndrome, T2D and hypertension. According to Lang *et al.* (2012), the relative risk of metabolic syndrome is 1.33 for one exposure period to job stress and 1.72 for a double exposure period, indicating a dose–effect relationship.

Cardiovascular disease, as a major complication of T2D and obesity, is closely influenced by working environment on the health of the professionals (Lang *et al.*, 2012). An unstable situation, whether it involves unemployment or the fear of losing a job, has various effects on cardiovascular health. These effects may also impact those in other types of unstable employment, such as fixed-term contracts, involuntary part-time work, seasonal work and internships.

Leclerc *et al.* (2008) reported that in the long term, the strategy for preventing CVD, especially in diabetic patients, should not only be designed to be applied "within" the family and professional environment, but "upon" this environment, on the individual and their reactions. "Stress management" courses should be offered to employees. This constitutes an issue of the interaction between an employee and his environment.

Via the questionnaire we evaluated sport exercise, defined as a subset of physical activity that is planned, structured, and repetitive with final or intermediate objectives of the improvement or maintenance of physical fitness.

Physical activity, in terms of exercise in the prevention and management of obesity, should focus on endurance (or cardiovascular/aerobic) modes of exercise (Cosme *et al.*, 2015). Likewise, physical activity can help people with diabetes achieve a variety of goals, including increased cardiorespiratory fitness, increased vigour, improved glycemic control, decreased

insulin resistance, improved lipid profile, blood pressure reduction and maintenance of weight loss (Colberg*et al.*, 2010; Chudyk&Petrella, 2011).

About 24% of our patients (three groups) practiced regularly aerobic exercises.Those exercises are defined by the Canadian Diabetes Association (CDA) as physical activities including walking, cycling or jogging, that involves continuous, rhythmic movements of large muscle groups lasting for at least 10 minutes at a time (CDA, 2013).The practice of sport in our three groups of patients remained far from the recommendations whether for number of patients who practiced as well as the weekly frequency for those who practiced sport. According to Chudyk&Petrella, 2011 and Umpierre*et al.*, 2011 people with diabetes should accumulate a minimum of 150 minutes of moderate- to vigorous-intensity aerobic exercise every week, spread over at least 3 days of the week, with no more than 2 consecutive days without exercise.

Regarding family antecedents, our results show an agreement with literature, either for obese patients without diabetes or T2D ones (the two groups of diabetic patients), hereditariness and co-morbidities are among risk factors for the onset of obesity and diabetes (ADA, 2004; Otero *et al.*, 2007).

The assessment of food habits is pivotal in the dietary management of T2D and obesity. It is well known that because assessment of dietary intakes is generally obtained by self-reported records and declaration, they are subject to a number of reporting biases, which may lead to misrepresentation of current intake and therefore compromise the validity of the data. The issue of energy intake underreporting and diabetes was first raised by Prentice *et al.* (1993) and has since been discussed in only a few studies conducted in T2D patients (Sallé*et al.*, 2006).

Our results showed that overweight/obese diabetic patients had the highest energy intake comparing to the two other groups.These findings agree with those of Visockienė*et al.*, 2006 and Sallé*et al.*, 2006. Gender did not influence the level of energy reporting, with males and females reporting to a similar degree.

In whole groups, the lunch was the most important daily meal that brought the highest amount of calorie intake. This result is in compatibility with Tounian (2006) findings, in the Mediterranean region, where lunch remains the main meal. However, there is a shift towards the dinner, according to the organization of the working day. In the southern and eastern Mediterranean region, we "go home" whenever possible for lunch. The reason is the attachment to tradition and family. Indeed, in our investigation dinnerwas the most respected meal by all patients in the tree groups.

Ashwal&Hod (2015)have suggested that lunch and dinner should each account for about 30% of the daily calorie intake, and the rest should be distributed as snacks throughout the day. On the total, the diet should consist of three meals a day and some snacks. The regimen of smaller, frequent meals lead to better satiety and compliance, with a reduction of postprandial glucose peaks. Findings of the present study showed an exceeding of calorie contribution of lunch and dinner versus a weak contribution of breakfast in the daily energy gained in all patients of both genders.

Regarding the comparison between main energy nutrients within the three groups, carbohydrates and proteins didn't reveal any significant differences. However, we noticed a difference of fat consumption. This result matches Visockienė*et al.*, 2006 findings.

The assessment of carbohydrates (monosaccharides, disaccharides and polysaccharides) intake did not show significant difference between the two genders within all groups. Some large observational studies have provided conflicting results, showing both positive and

negative associations of total carbohydrate intake with diabetes and obesity risk (Hodge *et al.*, 2004; Park *et al.*, 2010).Instead, glucose levels are determined by the quality of carbohydrates ingested, which influence on gastrointestinal transit and the velocity of nutrient absorption, and the long-term risk of diabetes (Buyken *et al.*, 2010). Four important qualitative features of dietary carbohydrates relevant to diabetes are fibre, wholegrain seeds, glycemic index (GI), and simple sugars in beverages.

In our results, assessment of saturated fatty acids (SFA) revealed significant differences between the three patients groups for both medium- and long chain SFA.

Overweight/obese diabetic patients (both genders) had the highest levels of dietary SFA intake followed by overweight/obese non diabetic patients. Phillips *et al.* (2012) suggested that genetic predisposition to obesity may be modulated by dietary SFA intake. This may be particularly relevant to individuals with diet-related metabolic disease who are at increased cardiometabolic risk. Likewise, dietary SFA consumption modulated the relationship between "fat mass and obesity-associated protein" gene (*FTO*) *rs9939609* and waist circumference. Overweight and obesity are accentuated among high-SFA consumers. These results may give a satisfactory explanation for our findings. However, the potential role of SFA intake in the development of diabetes is also in line with data from the ULSAM cohort (Risérus *et al.*, 2007).

The outcomes of our investigation about monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids(PUFA) indicated that for both genders, the overweight/obese diabetic patients group had the higher dietary intake of MUFA and PUFA followed by overweight/obese non-diabetic patients then the normal weight diabetic ones. The beneficial effect of diets rich in MUFA and/or polyunsaturated fatty acids has been shown in several studies. In KANWU study, and with the content total fat of 38%, impaired insulin sensitivity

was reported in those who consumed a SFA-enriched diet compared with those who consumed a MUFA-rich diet (Pérez-Jiménez *et al.*, 2001). The same findings were reported by Paniagua and colleagues (2007) with a short intervention period (28 days) and a relatively high intake of total fat.

In a randomized trial by Summers *et al.* (2002), a beneficial effect of diet rich in PUFA *n*-6 (e.g., linoleic acid) improved insulin sensitivity when compared with a SFArich diet after only 5 weeks, the study was conducted on a sample that included over than 50% of obese and/or diabetic participants. Another significant finding of their study was the reduction of visceral fat when SFA was replaced by PUFA.

According to Madigan *et al.* (2000), comparing between linoleic acid (18:2), as a parent acid of *n*-6 series (PUFA), and oleic acid (18:1) (MUFA), suggest that linoleic acid rich diet may not be the best option for people with T2D. A linoleic acid–rich diet was associated with increased fasting insulin and glucose levels, increased postprandial lipoproteins, and significantly higher plasma and LDL-c levels, all of which are associated with atherosclerosis risk. However, an oleic acid–rich diet appears to be a more suitable option for T2D patients.

Regarding the assessment of amino acids intake, our results did not show any significant differences neither between the three groups of patients or between males and females within each group. Amino acids are very important dietary components that are not only necessary for the synthesis of proteins, but they have other metabolic functions and roles in ameliorating or restoring metabolic imbalance and in ameliorating muscle catabolism.

Our findings showed that amino acids intake, in the three groups of patients, were in accordance with the highest median intake of essential amino acids recommended by the *Institute of Medicine* (2011). There are clinical evidences suggesting that even the dietary

intake or supplementations of essential amino acids, especially branched chain amino acids (Leucine, Isoleucine and Valine), have beneficial effects on body weight, body fat, lean body mass, and insulin sensitivity (Rajkumar*et al.*, 2015). Furthermore, the population-based International Study of Macro-/Micronutrients and Blood Pressure (INTERMAP) provided unique evidence to evaluate the effects of dietary branched chain amino acids (BCAAs). Higher dietary BCAA intake was associated with a lower prevalence of overweight and obesity in middle-aged individuals from East Asian and Western countries (Qin *et al.*, 2011).

Dietary intake of essential amino acids in the three patients groups does not seem to show any deficiencies, which is in favor of our patients. However, a supplementation in branched chain amino acids may provide further positive improvements for obese and/or T2D patients.

As nutrients, vitamins and minerals play diverse roles in our bodies. Micronutrients can regulate metabolism and gene expression and influence the development and progression of many chronic diseases.Vitamins are vital to cardiovascular health (i.e. vitamin B_1), nerve function (ie, vitamins B_6 and B_{12}), the production of red blood cells (i.e. vitamins B9 and B_{12}), and coagulation (ie, vitamin K), among many other functions (Christie-David*et al.*, 2015).

Despite the fact that micronutrient requirements can be difficult to determine because many assessment methods are noninvasive and dietary assessment methods based on self-declaration of the patient himself as well as databases are not perfectly accurate (O'Connell, 2001). Results of the present study indicated a very low dietary intake of vitamins D and B_9 and some minerals (calcium, potassium, magnesium and iodine) in all patients of both genders. However, we noticed also a low dietary intake in B group vitamins such as: B_1, B_5 and B_{12}, in normal weight diabetic patients.

Some studies have found an association between vitamin D contribution and both obesity and insulin resistance. Although suggestive, these observational studies did not prove a causal relationship between vitamin D and insulin resistance. A number of factors may confound this relation, such as adiposity. Obese individuals may avoid sun exposure, and vitamin D (a lipophilic compound) may be trapped in adipose tissue, resulting in serum deficiency (Valcour*et al.*, 2012).

The relationship between vitamin D and calcium has been discussed in several studies. Vitamin D can change the intracellular calcium signals and plays a role in the secretion of pancreatic insulin and insulin sensitivity, both of which relate to calcium levels. Moreover, the role of calcium in the development of T2D has been indirectly suggested by cross-sectional studies in which a high calcium intake has been found to be inversely associated with body weight and adiposity (Jacqmain*et al.*, 2003).

Some studies reported that improvement in the vitamin D status of T2D patients is beneficial for glycemic optimization and weight control. Likewise, dietary supplementation in vitamin D and calcium together improves lipid profile in dyslipidemic subject and plays a preventive role against overweight risk (Heaney*et al.*, 2002; Nikooyeh*et al.,* 2011; Christie-David*et al.*, 2015).

The usual diet of diabetic patients and/or obese in the three groups is unable to satisfy the mineral and vitamin needs. It is clear that low-calorie diets usually involve deficits in vitamins and mineral, especially in obese subjects to which vitamin supplementation is still under discussion (ADA, 2006).Considering that anti-diabetic (especially metformin) treatment is associated with decrease in serum vitamins B_{12} and B_9 of 16 to 22% (Aarsand&Carlsen, 1998; Wulffelé*et al.*, 2003), we should encourage people with diabetes to

meet their daily needs by eating in balanced way. The use of vitamin or mineral supplements is suitable only when the food consumption become very inadequate.

As an important purpose of the present study, we evaluated, during both fasting and postprandial states, glucose level, lipids blood parameters, and dyslipidemia (identified through lipid and apolipoproteins ratios), in all patients including both genders.

Our results, in the two groups of diabetic patients (either overweight/obese or normal weight), indicated a higher fasting and postprandial glucose levels in men comparing to women. These findings disagree with the results of DECODE study (2003) which reported age- and sex-effect on glucose levels in 13 cohorts from nine European countries. DECODE study results indicated that after 50years of age, diabetic women had significantly higher means of 2 hours postprandial glucose than men (Qiao*et al.*, 2003).However, results from middle-east region of Isfahan Diabetes Prevention Study, conducted on 2368 T2D patients, indicated a higher postprandial glucose in males than females (Janghorbani&Amini, 2008).

In their study, Cavalo*et al.* (2011) followed 505 of individuals with diabetes for about 14 years and assessed the relationship of postprandial glycemia with mortality. Hyperglycemia two hours after lunch was identified as an independent risk factor for cardiovascular mortality and overall mortality.

In non-diabetic overweight/obese patients, males had the higher levels of glucose than women. The same results were reported by Song *et al.* (2014) in their study; "Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe" based on 11 cohorts and including 45 594 individuals.

Regarding lipids, the evaluated parameters (total cholesterol, HDL-c, LDL-c, and triglycerides), did not show significant differences between the two genders during fasting

orpostprandial states, with exception to postprandial HDL-c in overweight/obese patients with diabetes (p=0.029) or without diabetes (p=0.048).

We noticed an increase in total cholesterol, LDL-c and triglycerides during the 3 to 4 hours after meal consumption with an HDL-c decrease in all patients of both genders. Our findings are different from results found by Ntyintyane*et al.* (2008) and Langsted*et al.* (2008), in which only triglycerides showed a postprandial positive increase. Contrariwise, Coutinho*et al.* (2008) found that, in addition to an increase in TG levels, consumption of a test meal rich in fat was associated with an increase in blood leukocytes and a decrease in HDL-c.

Fasting triglycerides in our overweight/obese patients were above 1.50 g/ml, thispositive relationship between overweight/obesity and the increaseof fasting TG levels corroborateswith previous and recent studies (Cercato*et al.*, 2004; Bansal*et al.*, 2007; Nogaroto*et al.*, 2015).

No substantial evidence demonstrated that fasting lipid levels are superior to nonfasting levels for cardiovascular risk prediction. Therefore, more arguments about the main reasons for measuring lipid levels in the fasting rather than the non fasting state are simply that it has become the norm worldwide and that the fasting requirement has been applied in almost all randomized lipid-lowering trials. However, since the works of Stampfer*et al.* (1996) who showed that plasma TG levels measured 3 to 4 hours after a meal were better than fasting plasma TG levels in predicting future cases of myocardial infarction, more recent studies confirming the same suggestion are available.

On the other hand, the comparison of HbA1c, apo A1 and apo B levels between the two genders did not show any significant differences within the three patients groups. Nevertheless, all these parameters were higher than required values (ADA, 2014; Jellinger*et*

al., 2012). In the same context, as reported by Elley*et al.* (2008), HbA1c is considered as an important indicator of blood glucose control, an HbA1c "threshold" of 7% is a significant higher risk of macrovascular disease in diabetic patients. Every 1% higher HbA1c level above that threshold is associated with an independent increased CVD risk.

The risk of vascular disease is 2 to 4 fold greater in adults with diabetes than that of non-diabetic individuals (Roglic*et al.*, 2005). However, association between diabetes and risk factors for CVD such as dyslipidemia (previously called hyperlipemia), hypertension and hyperinsulinemia bring out the necessity of an aggressive management. There is now more evidence from cohort and meta-analysis studies suggesting that lipid ratios (TC/HDL-c, TG/HDL-c and LDL-c/HDL-c) have higher association with CVD than individual lipids (Ingelsson*et al.*, 2007; Lewington*et al.*, 2007; Genest*et al.*, 2009; Zhao *et al.*, 2014).

On the other hand, prospective risk studies suggest that the use of apo B/ apo A-I ratio may be a promising and a convenient marker of risk of CVD (e.g. AMORIS study (Walldius*et al.*, 2001), INTERHEART study (Yusuf *et al.*, 2004), EPIC-Norfolk study (Van der Steeg*et al.*, 2007), and ULSAM study (Dunder*et al.*, 2004)).

The results of this study clearly showed, for both genders, increases in all lipid ratios during both fasting and postprandial states comparing to the requirements established by Pereira (2012). These results reflect a high risk in all of our patients.

Although no significant differences in lipid ratio were found between males and females. High total cholesterol/HDL-c values were noticed in males comparing to females in the three groups of patients. Consistent with this result, many studies demonstrated (whether in obese, diabetic or healthy population) that male gender is correlated with a high total cholesterol/HDL-c ratio comparing to female gender(Steptoe &Brydon, 2005; kanniyappan*et al.*, 2011; Gasevic*et al.*, 2014).However, TG/HDL-c values were elevated in

diabetic women comparing to men but it was higher in obese men comparing to obese women. According to Wägner*et al.* (2005), patients with diabetes often have an abnormally high number of small dense LDL particles, which has been found to be related to the TG/HDL-c ratio. The value of TG/HDL-c ratio takes into account both factors, i.e. the lipid load and the ability to remove the lipid laden particle from the circulation. The TG/HDL-c ratio gives an assessment of the total integrated lipid exposure to the tissues and it allows differentiating those subjects who are at greater risk of CHD (Khan *et al.*, 2008). Nevertheless, TG/HDL-c was shown not to be a reliable risk marker in individuals living in South Asia (Gasevic*et al.*, 2012) and African American people (Giannini*et al.*, 2011).

Results of prospective studies also suggest that a high LDL-c/HDL-c ratio combined with hypertriglyceridemia is associated with highest CHD risk. This dyslipidemic state (lipid triad) has been described as atherogenicdyslipidemia (Grundy, 1997).

Our findings showed no significant differences between males and females regarding apolipoproteins ratios. Furthermore, after adapting our data with the risk ranges of AMORIS (Walldius*et al.*, 2001) and INTERHEART (Yusuf *et al.*, 2004) studies, our patients are considered prone to cardiovascular risk (myocardial infarction) especially diabetic patients (whether obese or not).

As an important feature for using apo A-I, apo B and apo B/apo A-I ratio is that apolipoproteins concentrations are not affected by meals and are slightly influenced by biological variables, unlike the ordinary lipid parameters which fluctuate widely depending to food intakes. Therefore, the measurements of the apolipoproteins do not require fasting blood samples (Tietz, 1987; Walldius*et al.*, 2001; Shilpasree*et al.*, 2013; Tamang*et al.*, 2014). In clinical practice, apolipoproteins A-I and B may be measured directly in plasma without

noticeable interference with high triglyceride levels using accurate and precise internationally standardized methods (Tietz, 1987; Marcovina& Packard, 2006).

Mutual associations between apo B/apoA-I during both fasting and postprandial stages indicated that apolipoproteins ratio (apo B/apo A-I) was more correlated with postprandial TC/HDL-c (r^2= 0.298, p<0.001, F=120.352) and postprandial LDL-c/HDL-c (r^2= 0.484, p<0.001, F=86.632) and lightly correlated with postprandial TG/HDL-c (r^2=0.121, p<0.001, F=39.076). Similar results were presented in other studies (Wang *et al.*, 2005; Khan *et al.*, 2007; Taskinen*et al.*, 2010; Belfki*et al.*, 2011; Yan *et al.*, 2012; Hou*et al.*, 2013; Salazar *et al.*, 2014).

Although the vast majority of patients with established T2D should be considered at high short-term risk, the association of the diabetic disease with other risk factors such as overall and/or visceral obesity should lead to intensive monitoring and management of this situation.

Conclusion

Conclusion & Recommendations

A higher risk of vascular disease, including cardiovascular diseases, atherosclerosis, myocardial infarction, stroke, and peripheral vascular disease, is generally correlated with T2D and/or obesity in adults. That is because people with T2D often have obesity, hypertension, abnormal lipemia, lack of physical activity and poorly controlled blood sugars. However, by monitoring all these risk factors, diabetes patients may avoid or delay the development of heart and blood vessel disease. At the end of our investigation, which aimed to evaluate the postprandial glucose and lipids metabolism state in obese people with T2D, the gathered data allow to draw some conclusions that might help researchers and health care professionals to manage correctly the postprandial state among people with T2D:

- There is not a huge gender effect on postprandial metabolic responses. However, avoiding postprandial responses abnormalities requires a good mastering of body weight taking into consideration the physiological differences between males and females;

- Most patients with T2D of both genders, whether normal weight, overweight or obese, have dyslipidemia to varying degrees;

- Compared with individual lipid parameters, the changes of lipid ratios could reflect impaired lipid metabolism at earlier stage. However, lipid ratios were often influenced by food intake that could make interpretation of results more difficult depending on fasting or postprandial stages;

- On the basis of lipid ratios, people with T2D have a greater chance of developing serious complications and health issues, especially if their disease is associated with

other risk factors like overweight/obesity, abdominal fat accumulation and increased levels of HbA1c;

- Non-obese patients with T2D have a similar increased risk of cardiovascular disease, identified according to lipid and apolipoproteins ratios, as obese patients with T2D;
- The use of apolipoproteins can be of great practical advantage for patients and physicians. Apolipoproteins can be measured on non-fasting samples. Furthermore, it is more interesting to use a single ratio as risk prediction tools instead of referring to a larger number of lipid ratios.

Health risk assessment strategy including cardiovascular risk in prediabetic individuals such as obese or diabetic patients should not minimize terms of compliance, adherence, concordance, and persistence, all indicate different aspects of a person's relationship with his or her disease.

Continuity and discontinuity in the management of chronic diseases such as obesity and diabetes are very important concepts. If continuity of care, in diabetes, is easier said than done, discontinuity reasons may be an unpleasant encounter with a health care professional; his or her inability to explain the cause or course of symptoms or side effects, unwillingness to craft a person-friendly regime suited to individual needs and preferences, or difficulty in effecting mid-course corrections if an initial regime has not provided desired results. Likewise, lack of accessibility of treatment or lack of integrated health care services providing multidisciplinary care can also lead to discontinuity.

Care management should be centered on the relationship between the diabetic disease and its co-morbidities such as overweight, obesity, hypertension, hyperglycemia and dyslipidemia considered as risk factors for cardiovascular disease events. The prevention

strategy must focus upon a larger picture and includes all stakeholders such as health care organizations, the family and the community in its ambit.

Bibliography

Bibliography

AACE (American Association of Clinical Endocrinologists) Medical Guidelines for Clinical Practice for the Management of Diabetes Mellitus, Endocr Pract, 2007; 13:5-68.

Aarsand AK, Carlsen SM. Folate administration reduces circulating homocysteine levels in NIDDM patients on long-term metformin treatment. J Intern Med. 1998; 244(2): 169-174.

Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care. 2006; 29(5):1130-1139.

Abrahamson MJ. Optimal Glycemic Control in Type 2 Diabetes:Mellitus Fasting and Postprandial Glucose in Context. Arch Intern Med. 2004; 164(5): 486-491.

ACE (American College of Endocrinology) Consensus statement on guidelines for glycemic control. Endocr Pract. 2002; 8 (Suppl 1):5-11.

ADA (American Diabetes Association) Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2011; 34 Suppl 1: S62-9.

ADA (American Diabetes Association) Evidencebased nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. Diabetes Care. 2002; 25: 202-212.

ADA (American Diabetes Association) Executive Summary: Standards of Medical Care in Diabetesd. Diabetes Care. 2014; 37(Supp1): s5-s13.

ADA (American Diabetes Association) Screening for type 2 diabetes. Diabetes Care. 2004; 3(2):60-63.

ADA (American Diabetes Association) Standards of medical care for patients with diabetes mellitus. Diabetes Care. 2001; 24(suppl 1): S33-S55.

ADA (American Diabetes Association) Standards of Medical Care in Diabetes-2007. Diabetes Care. 2007; 30 (Suppl 1): S1-S41.

ADA (American Diabetes Association) Standards of medical care in diabetes-2013. Diabetes Care. 2013; 36(suppl 1): S11-S66.

ADA (American Diabetes Association) Standards of medical care in diabetes-2014. Diabetes Care. 2014; 37(suppl 1): S14-S80.

ADA (American Diabetes Association) Standards of Medical Care in Diabetes - 2006, Diabetes Care, (2006); 29 (1): S4-S42.

Adiels M, Olofsson SO, Taskinen MR, Boren J. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol. 2008; 28: 1225-1236.

Air EL, Strowski MZ, Benoit SC, Conarello SL, Salituro GM, Guan XM, Liu K, Woods SC, Zhang BB. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nature Med. 2002; 8, 179-183.

Alberti KG, Zimmet P, Shaw J. Metabolic syndrome-a new world-wide definition. A consensus statement from the International Diabetes Federation. Diabet Med. 2006; 23(5):469-80.

Alberti, KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Provisional report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Diabet Med. 1998;15(7):539-553.

Alexander CM, Landsman PB, Teutsch SM, Haffner SM. Third National Health and Nutrition Examination Survey (NHANES III); National Cholesterol Education Program (NCEP). NCEPdefined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes. 2003; 52:1210-1214.

Alipour A, Elte JWF, Rietveld AP, van Zaanen HCT, Castro Cabezas M. Postprandial inflammation and endothelial dysfunction. Biochem Soc Trans. 2007; 35(Pt 3): 466-469.

Al-Moosa S, Allin S, Jemiai N, Al-Lawati J, Mossialos E. Diabetes and urbanization in the Omani population: an analysis of national survey data. Popul Health Metr. 2006; 4: 5.

Amrane N, Khaled MB. Effect of Overweight and Obesity on Postprandial Lipemia among the Subjects with Type 2 Diabetes. J Diabetes Metab. 2012; 3:178.

Anderson RM. Applied principles of teaching and learning. In: A Core Curriculum for Diabetes Education: Diabetes education and program management. 4th ed. Chicago: American Association of Diabetes Educators; 2001. P.1-18.

Aronne LJ, Nelinson DS, Lillo JL. Obesity as a disease state: a new paradigm for diagnosis and treatment. Clin Cornerstone. 2009; 9(4):9-25.

Ashwal E, Hod M. Gestational diabetes mellitus: Where are we now?. Clin Chim Acta. 2015 Feb 2. pii: S0009-8981(15)00043-1.

Avignon A, Radauceanu A, Monnier L. Nonfasting plasma glucose is a better marker of diabetic control than fasting plasma glucose in type 2 diabetes. Diabetes Care. 1997; 20: 1822-1826.

Axelsen M, Eliasson B, Joheim E, Lenner RA, Taskinen MR, Smith U. Lipid intolerance in smokers. J Intern Med.1995; 237: 449-455.

Baggio LL, Huang QL, Brown TJ, Drucker DJ. A recombinant human glucagon-like peptide (GLP)-1-albumin protein (Albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. Diabetes. 2004; 53: 2492-2500.

Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA. 2007; 298(3): 309-316.

Barsh GS & Schwartz MW. Genetic approaches to studying energy balance: perception and integration. Nature Rev. Genet. 2002 ; 3, 589-600.

Barter PJ, Ballantyne CM, Carmena R, Castro Cabezas M, Chapman MJ, Couture P, de Graaf J, Durrington PN, Faergeman O, Frohlich J, Furberg CD, Gagne C, Haffner SM, Humphries SE, Jungner I, Krauss RM, Kwiterovich P, Marcovina S, Packard CJ, Pearson TA, Reddy KS, Rosenson R, Sarrafzadegan N,Sniderman AD, Stalenhoef AF, Stein E, Talmud PJ, Tonkin AM, Walldius G, Williams KM. ApoB versus

cholesterol to estimate cardiovascular risk and to guide therapy: Report of the thirty-person ten-country panel. J Intern Med. 2006; 259: 247-258.

Beaumier-Gallon G, Dubois C, Senft M, Vergnes MF, Pauli AM, Portugal H, Lairon D. Dietary cholesterol is secreted in intestinally derived chylomicrons during several subsequent postprandial phases in healthy humans. Am J Clin Nutr. 2001; 73(5): 870-877.

Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA. 2002; 287: 2570-2581.

Belfki H, Ben Ali S, Bougatef S, Ben Ahmed D, Haddad N, Jmal A, Abdennebi M, Ben Romdhane H. The Apolipoprotein B/Apolipoprotein A 1 ratio in relation to metabolic syndrome and its components in a sample of the Tunisian population. Exp Mol Pathol. 2011; 91(2): 622-625.

Bell GC, Walley AJ, Froguel P. The genetics of human obesity. Nature Reviews Genetics. 2005; 6: 221-234.

Berry SE, Miller GJ, Sanders TA. The solid fat content of stearic acid-rich fats determines their postprandial effects. Am J Clin Nutr. 2007; 85: 1486-1494.

Bjorntrop P. Do stress reactions cause abdominal obesity and comorbidities. Obes Rev 2001; 2: 73-86.

Bonen A, Dohm Gl, van Loon LJ. Lipid Metabolism, Exercise and Insulin Action. Essays Biochem. 2006; 42: 47-59.

Boulé NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA. 2001; 286: 1218-1227.

Bravo E, Mariarosaria Napolitano M, Botham KM. Postprandial Lipid Metabolism: The Missing Link Between Life-Style Habits and the Increasing Incidence of Metabolic Diseases in Western Countries?. The Open Translational Medicine Journal. 2010; 2: 1-13.

Bray GA. The Metabolic Syndrome and Obesity. Humana Press Inc., a part of Springer Science and Business Media, (2007); pp 303.

Brender JR, Hartman K, Nanga RP, Popovych N, de la Salud BR, Vivekanandan S, Marsh EN, Ramamoorthy A. Role of zinc in human islet amyloid polypeptide aggregation. J Am Chem Soc. 2010, 132(26): 8973-8983.

Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4-day period. Obesi (Silver Spring) 2010; 18: 1176-1182.

Brownlee M. The pathobiology of diabetic complications. A unifying mechanism. Diabetes. 2005; 54: 1615-1625.

Brunzell JD, MICHAEL Davidson M, Furberg CD, Goldberg R, Howard BV, Stein JH, Witztum JL. Lipoprotein Management in Patients With Cardiometabolic Risk. Diabetes Care. 2008; 31(4): 818-822.

Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ. Primary prevention of cardiovascular diseases in people with diabetes mellitus. Diabetes Care. 2007; 30: 162-172.

Buyken AE, Mitchell P, Ceriello A, Brand-Miller J. Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. Diabetologia. 2010; 53(3): 406-418.

CADTH (Canadian Agency for Drugs and Technologies in Health) Second-line therapy for patients with type 2 diabetes inadequately controlled on metformin: a systematic review and cost-effectiveness analysis. Ottawa: The Agency. Optimal therapy report. 2010; 4 (2).

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003; 348(17): 1625-1638.

Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004; 84:277-359.

Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ. Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong

relationship in subjects at both low and high risk of NIDDM. Diabetes. 1996; 45:633-638.

Carrel G, Giusti V. Prise alimentaire : pas uniquement une question de volonté !. Rev Med Suisse. 2009; 5: 769-772.

Castañeda TR, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. Frontiers in Neuroendocrinology. 2010; 31: 44-60.

Cavalot F, Pagliarino A, Valle M, Di Martino L, Bonomo K, Massucco P, Anfossi G, Trovati M. Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga Diabetes Study. Diabetes Care. 2011; 34(10): 2237-2243.

CDA (Canadian Diabetes Association) Clinical Practice Guidelines Expert Committee. Canadian Diabetes Association 2013 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada. Can J Diabetes. 2013; 37(suppl 1): S1-S212.

CDA (Canadian Diabetes Association) Dyslipidemia in Adults With Diabetes. Clinical Practice Guidelines Expert Committee. Canadian Journal Of Diabetes. 2006; 30(3):230-240.

CDC (Centers for Disease Control and Prevention) National Diabetes Fact Sheet: General Information and National Estimates on Diabetes in the United States. Atlanta, (2005).

Cercato C, Mancini MC, Arguello AMC, Passos VQ, Villares SMF, Halpern A. Systemic hypertension, diabetes mellitus, and dyslipidemia in relation to body mass index: evaluation of a Brazilian population. Rev. Hosp. Clin. 2004; 59(3):113-118.

Ceriello A, Colagiuri S. Review of IDF guideline for management of postmeal glucose. Diabet Med. 2008;25:1151-1156.

Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes. 2005; 4: 1-7.

Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin : more than just another fat cell hormone ? Diabetes Care. 2003; 26: 2442-2450.

Christie-David DJ, Girgis CM, Gunton JE. Effects of vitamins C and D in type 2 diabetes mellitus. Nutrition and Dietary Supplements. 2015; 7: 21-28.

Chudyk A., Petrella RJ. Effects of exercise on cardiovascular risk factors in type 2 diabetes: a meta-analysis. Diabetes Care. 2011; 34(5): 1228-1237.

Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B. Exercise and Type 2 Diabetes : The American College of Sports Medicine and the American Diabetes Association: joint position statement. Diabetes Care. 2010; 33(12): e147–e167.

Cosme F. Buzzachera , Marco Meucci , and Carlo Baldari . Physical Activity and Training Prescription. In ; Lenzi Andrea, Silvia Migliaccio, Lorenzo Maria Donini. Multidisciplinary Approach to Obesity From Assessment to Treatment. Springer International Publishing Switzerland, (2015): p. 253.

Couch SC, Isasi CR, Karmally W, Blaner WS, Starc TJ, Kaluski D, Deckelbaum RJ, Ginsberg HN, Shea S, Berglund L. Predictors of postprandial triacylglycerol response in children: The Columbia University Biomarkers Study. Am J Clin Nutr. 2000; 72: 1119-1127.

Coutinho ER, Macedo GM, Campos FS, Bandeira FA. Changes in HDL cholesterol and in the inflammatory markers of atherogenesis after an oral fat load in type-2 diabetic patients and normal individuals. Metab Syndr Relat Disord. 2008; 6(2):153-157.

Cox-York KA, Sharp TA, Stotz SA, Bessesen DH, Pagliassotti MJ, Horton TJ. The effects of sex, metabolic syndrome and exercise on postprandial lipemia. Metabolism. 2013; 62(2): 244-254.

Cryer PE. Current concepts: Diverse causes of hypoglycemia-associated autonomic failure in diabetes. N Engl J Med. 2004; 350: 2272-2279.

Czernichow S, Mennen L, Bertrais S, Preziosi P, Hercberg S, Oppert JM. Relationships between changes in weight and changes in cardiovascular risk factors in middle-aged French subjects: effect of dieting. Int J Obes Relat Metab Disord. 2002; 26(8):1138-1143.

Day C, Bailey CJ. Obesity in the pathogenesis of type 2 diabetes. British Journal of Diabetes & Vascular Disease. 2011; 11: 55.

DCCT (Diabetes Control and Complications Trial) Research Group: Hypoglycemia in the Diabetes Control and Complications Trial. Diabetes. 1997; 46: 271–286.

DCCT/EDIC (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005; 353: 2643–2653.

De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism and relation to premature atherosclerosis. Eur J Clin Invest. 1996; 26(2): 89–108.

Derosa G, Mugellini A, Ciccarelli L, Fogari R. Randomized, double-blind, placebo-controlled comparison of the action of orlistat, fluvastatin, or both an anthropometric measurements, blood pressure, and lipid profile in obese patients with hypercholesterolemia prescribed a standardized diet. Clin Ther. 2003; 25: 1107–1122.

DHHS (Departement for Health and Human Services) Dietary guidelines for Americans. 6th ed. Washington, DC: The National Academies Press, 2005.

Diamond J. The double puzzle of diabetes. Nature. 2003; 423 : 599–602.

Drewnowski A. Nutrition transition and global dietary trends. Nutrition. 2000; 16(7–8): 486–7.

Drucker DJ. The biology of incretin hormones. Cell Metab.2006; 3:153–165.

Drucker DJ. The role of gut hormones in glucose homeostasis. J Clin Invest.2007; 117:24–32.

Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM, Bernard PM, Latgé C, Lafont H, Borel P, Lairon D. Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. Am J Clin Nutr. 1994; 60(3): 374–382.

Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sook-Sul H. Regulation of Lipolysis in Adipocytes. Annu. Rev. Nutr. 2007; 27:79–101.

Dunder K, Lind L, Zethelius B, Berglund L, Lithell H. Evaluation of a scoring scheme, including proinsulin and the apolipoprotein B/apolipoprotein A1 ratio, for the risk of acute coronary events in middle-aged men: Uppsala Longitudinal Study of Adult Men (ULSAM). Am Heart J. 2004; 148(4): 596-601.

EASD (European Association for the Study of Diabetes) The Diabetes and Nutrition Study. Recommendations for the nutritional management of patients with diabetes mellitus. Eur J Clin Nutr. 2000; 54: 353-355.

Edgerton DS, Cardin S, Emshwiller M, Neal D, Chandramouli V, Schumann WC, Landau BR, Rossetti L, Cherrington AD. Small increases in insulin inhibit hepatic glucose production solely caused by an effect on glycogen metabolism. Diabetes. 2001; 50(8): 1872-1882.

Einhorn D, Reaven GM, Cobin RH, Ford E, Ganda OP, Handelsman Y, Hellman R, Jellinger PS, Kendall D, Krauss RM, Neufeld ND, Petak SM, Rodbard HW,Seibel JA, Smith DA, Wilson PW. American College of Endocrinology position statement on the insulin resistance syndrome. Endocr Pract. 2003; 9(3): 237-252.

Ekblad E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides. 2002; 23:251-261.

Elbein SC, Hasstedt SJ, Wegner K, Kahn SE. Heritability of pancreatic beta-cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. J Clin Endocrinol Metab. 1999; 84 : 1398-403.

Elley CR, Kenealy T, Robinson E, Drury PL. Glycated haemoglobin and cardiovascular outcomes in people with Type 2 diabetes: a large prospective cohort study. Diabet Med. 2008; 25(11): 1295-1301.

Elmadfa I, Kornsteiner M. Fats and fatty acid requirements for adults. Ann Nutr Metab. 2009; 55(1-3): 56-75.

Emery-Tiburcio EE, Nackers LM, Bernfeld S, Lahey R, Lichtenberg PA, Mast BT, Carpenter BD, Loebach WJ, Diabetes and obesity in later life. In: APA handbook of clinical geropsychology, Vol. 2: Assessment, treatment, and issues of later life. Lichtenberg PA. (Ed); Mast BT. (Ed); Carpenter BD. (Ed); Loebach WJ (Ed). Washington, DC. (2015): pp 441-465.

English P, Williams G. Hyperglycaemic crises and lactic acidosis in diabetes mellitus. Postgrad Med J. 2004; 80:253-261.

Evert AB, Boucher JL, Cypress M, Dunbar SA, Franz MJ, Mayer-Davis EJ, Neumiller JJ, Nwankwo R, Verdi CL, Urbanski P, Yancy WS Jr. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care. 2013; 36(11): 3821-3842.

Felber JP, Ferrannini E, Golay A, Meyer HU, Theibaud D, Curchod B, Maeder E, Jequier E, DeFronzo RA. Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. Diabetes. 1987; 36(11): 1341-1350.

Fielding BA, Reid G, Grady M, Humphreys SM, Evans K, Frayn KN. Ethanol with a mixed meal increases postprandial triacylglycerol but decreases postprandial non-esterified fatty acid concentrations. Br J Nutr. 2000; 83: 597-604.

Filippatos T, Derdemezis C, Elisaf M. Effects of orlistat, alone or combined with hypolipidemic drugs, on cardiovascular risk factors. Clin Lipidol. 2009; 4: 331-341.

Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord. 1998; 22(1): 39-47.

Flier JS, Harris M, Hollenberg AN. Leptin, nutrition, and the thyroid: the why, the wherefore, and the wiring. J. Clin. Invest. 2000; 105, 859-861.

Fogal AS, Franceschini Sdo C, Priore SE, Cotta RM, Ribeiro AQ. Stature estimation using the knee height measurement amongst Brazilian elderly. Nutr Hosp. 2014; 31(2): 829-834.

Fong DS, Aiello LP, Ferris FL 3rd, Klein R. Diabetic retinopathy. Diabetes Care. 2004; 27: 2540-2553.

Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values 2002. Am J Clin Nutr. 2002; 76: 5-56.

Foster-Schubert KE, Alfano CM, Duggan CR, Xiao L, Campbell KL, Kong A, Bain CE, Wang CY, Blackburn GL, McTiernan A. Effect of diet and exercise, alone or

combined, on weight and body composition in overweight-to-obese postmenopausal women. Obesity (Silver Spring). 2012; 20(8): 1628-1638.

Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. Clinical Diabetes. 2008; 26 (2).

Freedland ES. Role of critical visceral adipose tissue threshold in metabolic syndrome: implications for controlling dietary carbohydrates: a review. Nutr Metab. 2004; 1: 12.

Fukuda S, Hirata A, Nishizawa H, Nagao H, Kashine S, Kimura T, Inoue K, Fujishima Y, Yamaoka M, Kozawa J, Kitamura T, Yasuda T, Maeda N, Imagawa A,Funahashi T, Shimomura I. Systemic arteriosclerosis and eating behavior in Japanese type 2 diabetic patients with visceral fat accumulation. Cardiovasc Diabetol. 2015; 14(1): 8.

Funnell MM, Brown TL, Weiss MA. National standards for diabetes self-management education. Diabetes Care. 2010; 33: S89-S96.

Gæde P,Vedel P, Larsen N, Jensen GVH, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med. 2003; 348:383-393.

Gao W, Qiao Q, Tuomilehto J. Post-challenge hyperglycaemia rather than fasting hyperglycaemia is an independent risk factor of cardiovascular disease events. Clin Lab. 2004;50 (9-10): 609-615.

Gasevic D, Frohlich J, Mancini GB, Lear SA. The association between triglyceride to high-density-lipoprotein cholesterol ratio and insulin resistance in a multiethnic primary prevention cohort. Metabolism. 2012; 61(4): 583-589.

Gasevic D, Frohlich J, Mancini GJ, Lear SA. Clinical usefulness of lipid ratios to identify men and women with metabolic syndrome: a cross-sectional study. Lipids Health Dis. 2014; 13: 159.

Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, Couture P, Dufour R, Fodor G, Francis GA, Grover S, Gupta M, Hegele RA, Lau DC,Leiter L, Lewis GF, Lonn E, Mancini GB, Ng D, Pearson GJ, Sniderman A, Stone JA, Ur E. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and

treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. Can J Cardiol. 2009; 25(10): 567-579.

Gervaise N, Garrigue MA, Lasfargues G, Lecomte P. Triglycerides, apoC3 and Lp B :C3 and cardiovascular risk in type 2 diabetes. Diabetologia. 2000; 43(6): 703-708.

Giannini C, Santoro N, Caprio S, Kim G, Lartaud D, Shaw M, Pierpont B, Weiss R. The triglyceride-to-HDL cholesterol ratio: association with insulin resistance in obese youths of different ethnic backgrounds. Diabetes Care. 2011; 34(8): 1869-1874.

Gibney MJ, Lanham-New SA, Cassidy A, Vorster HH. Introduction to Human Nutrition Textbook Series. The Nutrition Society. A John Wiley & Sons, Ltd., Publication. United Kingdom, (2009).

Gill JM, Frayn KN, Wootton SA, Miller GJ, Hardman AE. Effects of prior moderate exercise on exogenous and endogenous lipid metabolism and plasma factor VII activity. Clin Sci (Lond). 2001; 100(5): 517-527.

Ginsberg HN. Effects of statins on triglyceride metabolism. Am J Cardiol. 1998; 81(4A): 32B-35B.

Glasgow RE, Bull SS. Making a Difference With Interactive Technology: Considerations in Using and Evaluating Computerized Aids for Diabetes Self-Management Education. Diabetes Spectrum. 2001 ; 14(2) : 99-106.

Gonlachanvit S, Hsu CW, Boden GH, Knight LC, Maurer AH, Fisher RS, Parkman HP. Effect of Altering Gastric Emptying on Postprandial Plasma Glucose Concentrations Following a Physiologic Meal in Type-II Diabetic Patients. Digestive Diseases and Sciences. 2003; 48(3): 488-497.

Gotto AM. Management of dyslipidaemia. American Journal of Medicine. 2002 ; 112(8): 10-18.

Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, Granzotto M, Vettor R, Camastra S, Ferrannini E. Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. Diabetes. 2002; 51(1): 144-1451.

Greenberg AS, McDaniel ML. Identifying the links between obesity, insulin resistance and β-cell function : potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest. 2002; 32 (Suppl 3): 24-34.

Gropper SS, Smith JL. Advanced nutrition and human metabolism. Belmont, CA : Wadsworth/Cengage Learning, 2013; pp 586.

Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005; 28: 164-176.

Grundy SM, Cleeman JI, Bairey Merz CN, Bryan Brewer JrH, Clark LT, Hunninghake DB, Pasternak RC, Smith Jr SC, Stone NJ. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation. 2004; 110: 227-239.

Grundy SM. Small LDL, atherogenic dyslipidaemia, and the metabolic Syndrome. Circulation. 1997; 95(1):1-4.

Gupta A, Gupta R, Sarna M, Rastogi S, Gupta VP, Kothari K. Prevalence of diabetes, impaired fasting glucose and insulin resistance syndrome in an urban Indian population. Diabetes Res Clin Pract. 2003; 61(1): 69-76.

Hainer V, Toplak H , Mitrakou A. Treatment modalities of obesity: What fits whom? Diabetes Care. 2008; 31(suppl 2):S269-S277.

Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegelasch HJ, Lindner J. Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia. 1996; 39(12): 1577-1583.

Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes. 2004; 53 Suppl 1: S143-S151.

Heaney RP, Davies KM, Barger-Lux MJ. Calcium and weight: clinical studies. J Am Coll Nutr. 2002; 21(2): 152S-155S.

Heiat A, Vaccarino V, Krumholz HM. An evidencebased assessment of federal guidelines for overweight and obesity as they apply to elderly persons. Arch Intern Med. 2001;161:1194–1203.

Hisra A, Vikram NK. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. Nutrition. 2003; 19: 457–466.

Hodge AM, English DR, O'Dea K, Giles GG. Glycemic index and dietary fiber and the risk of type 2 diabetes. Diabetes Care. 2004; 27(11): 2701–2706.

Horowitz M, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. Diabet Med. 2002; 19: 177–194.

Horton TJ, Commerford SR, Pagliassotti MJ, Bessesen DH. Postprandial leg uptake of triglyceride is greater in women than in men. Am J Physiol Endocrinol Metab. 2002; 283: E1192–E1202.

Hou X, Lu J, Weng J, Ji L, Shan Z, Liu J, Tian H, Ji Q, Zhu D, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, Yang Z, Yang W, Jia W. Impact of waist circumference and body mass index on risk of cardiometabolic disorder and cardiovascular disease in Chinese adults: a national diabetes and metabolic disorders survey. PLoS One. 2013; 8(3): e57319.

Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004; 53(3):693–700.

Hui H, Tang G, W Go VL. Hypoglycemic herbs and their action mechanisms. Chinese Medicine 2009, 4: 1–11.

IDF (International Diabetes Federation) Clinical Guidelines Task Force, Guideline for management of postmeal glucose. Brussels. (2007): pp 29.

IDF (International Diabetes Federation) Diabetes Atlas. 5th ed. Brussels, Belgium, (2012).

Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, Pencina MJ, Schoonmaker C, Wilson PW, D'Agostino RB, Vasan RS. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA. 2007; 298(7): 776–785.

Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary

Reference Intakes for Calcium and Vitamin D. Washington (DC): National Academies Press (US); 2011.

Issa JS, Diament J, Forti N. Postprandial Lipemia: Influence of Aging. Arquivos Brasileiros de Cardiologia. 2005; 85(1).

Jackson KG, Knapper-Francis JM, Morgan LM, Webb DH, Zampelas A, Williams CM. Exaggerated postprandial lipaemia and lower post-heparin lipoprotein lipase activity in middle-aged men. Clin Sci (Lond). 2003; 105: 457-466.

Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. Am J Clin Nutr. 2003; 77(6): 1448-1452.

Jacqueminet S, Briaud I, Rouault C, Reach G, Poitout V. Inhibition of insulin gene expression by long-term exposure of pancreatic beta-cells to palmitate is dependent upon the presence of a stimulatory glucose concentration. Metabolism. 2000; 49(4): 532-536.

Janghorbani M, Amini M. Effects of gender and height on the oral glucose tolerance test: the isfahan diabetes prevention study. Rev Diabet Stud. 2008; 5(3): 163-170.

Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, Shepherd MD, Seibel JA. The AACE Task Force for Management of Dyslipidemia and Prevention of Atherosclerosis. AACE Lipid and Atherosclerosis Guidelines, Endocr Pract. 2012;18(Suppl 1) : 1-71.

Jimenez FL, S-Bergoderi MC. Obesity and the Heart. Rev Esp Cardiol. 2011;64(2): 140-149.

Jiménez-Gómez Y, Pérez-Jiménez F, Marín C, Gómez P, Moreno R, Delgado J, Pérez-Martínez P, Gómez MJ, López-Miranda J. The -250G/A polymorphism in the hepatic lipase gene promoter influences the postprandial lipemic response in healthy men. Nutr Metab Cardiovasc Dis. 2008 ;18(3):173-81.

Kaku K. Pathophysiology of Type 2 Diabetes and Its Treatment Policy. JMAJ. 2010; 53(1): 41-46.

Kannel WB. Risk stratification of dyslipidemia: Insights from the Framingham Study. Curr Med Chem Cardiovasc Hematol Agents. 2005; 3(3):187-193.

kanniyappan D, kalidhas P, Aruna RM. Age, gender related prevalence of cardiovascular risk factors in overweight and obese south Indian adults. Int J Biol Med Res. 2011; 2(2): 513-522.

Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N, Kandhro GA. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trace Elem Res. 2008; 122(1):1-18.

Kearney T, Dang C. Diabetic and endocrine emergencies. Postgrad Med J. 2007; 83:79-86.

Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a re-examination. Diabetes. 2000; 49(5): 677-683.

Kelley DE. Sugars and starch in the nutritional management of diabetes mellitus. Am J Clin Nutr. 2003; 78: 858S-864S.

Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond). 2008; 32(9): 1431-1437.

Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6): 2548-2556.

Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med. 2007; 7(1): 24-29.

Khan SR, Ayub N, Nawab S, Shamsi TS. Triglyceride profile in dyslipidaemia of type 2 diabetes mellitus. J Coll Physicians Surg Pak. 2008; 18(5): 270-273.

Kitabchi AE, Umpierrez GE, Murphy MB, Kreisberg RA. Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association. Diabetes Care. 2006; 29: 2739-48.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Diabetes Prevention Program Research Group. Reduction in the

incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002; 346: 393-403.

Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. Diabetes. 2003; 52, 948-956.

Korbints M. Obesity and Metabolism. Library of Congress Cataloging-in-Publication Data, (2008); 46-55.

Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes. 1998; 47: 859-866.

Kral JG, Management: Part III-Surgery. BMJ. 2006; 333 (7574): 900-903.

Kromhout D, Menotti A, Kesteloot H, Sans S. Prevention of coronary heart disease by diet and lifestyle: evidence from prospective cross-cultural, cohort, and intervention studies. Circulation. 2002; 105(7): 893-898.

Kyrou I, Valsamakis G, Tsigos C. The endocannabinoid system as a target for the treatment of visceral obesity and metabolic syndrome. Ann N Y Acad Sci. 2006; 1083: 270-305.

Lacroix A, Assal JP. L'éducation thérapeutique des patients: nouvelles approches de la maladie chronique. Maloine (2005): pp 240.

Lairon D, Lopez-Miranda J, Williams C. Methodology for studying postprandial lipid metabolism. European Journal of Clinical Nutrition. 2007; 61: 1145-1161.

Lairon D, Play B, Jourdheuil- Rahmani D. Digestible and indigestible carbohydrates: interactions with postprandial lipid metabolism. J Nutr Biochem. 2007; 18: 217-227.

Lairon D. Macronutrient uptake and modulation on chylomicron production and clearance. Atheroscler Suppl. 2008; 9: 45-48.

Lang T, Lepage B, Schieber AC, Lamy S, Kelly-Irving M. Social determinants of cardiovascular diseases. Public Health Reviews. 2012 ; 33(2) : 601-622.

Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008; 118(20): 2047-2056.

Laramee AS, Morris N, Littenberg B. Relationship of literacy and heart failure in adults with diabetes. BMC Health Serv Res. 2007; 7: 98.

LaRocque P, Mark Beard J, Mintz M. Type 2 Diabetes Prevention and Treatment. CME bulletin. 2009; 8(1).

Leahy JL. Pathogenesis of type 2 diabetes mellitus. Arch Med Res. 2005; 36(3): 197-209.

Leclerc A, Kaminski M, Lang T. Inégaux face à la santé: du constat à l'action. Editions La Découverte. Paris, (2008): p.298.

Lemieux S, Despres SP. Metabolic complications of visceral obesity: contribution to the etiology of life of type 2 diabetes and implications for prevention and treatment. Diabetes Metab. 1994; 20: 375-393.

Leung AM, Braverman LE. Consequences of excess iodine. Nature Reviews Endocrinology. 2014; 10: 136-142.

Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Arch Intern Med. 2004; 164(19): 2147-2155.

Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. Lancet. 2007; 370(9602): 1829-1839.

Lewis GF, Carpentier A, Adeli K, Giacca A. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev. 2002; 23:201-229.

Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett J. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation. 2006; 114(1): 82-96.

Lim CT, Kola B, Korbonits M. AMPK as a mediator of hormonal signalling. J Mol Endocrinol. 2010; 44(2): 87-97.

Lopez-Miranda J, Marin C. Dietary, Physiological, and Genetic Impacts on Postprandial Lipid Metabolism. In: Montmayeur JP, le Coutre J. (Ed). Fat Detection: Taste, Texture, and Post Ingestive Effects. CRC Press. Boca Raton (FL).2010.

Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr. 2007; 98: 458-473.

Lukic L, Lalic NM, Rajkovic N, Jotic A, Lalic K, Milicic T, Seferovic JP, Macesic M, Gajovic JS. Hypertension in obese type 2 diabetes patients is associated with increases in insulin resistance and IL-6 cytokine levels: potential targets for an efficient preventive intervention. Int J Environ Res Public Health. 2014; 11(4): 3586-3598.

Ma Y, Griffith JA, Chasan-Taber L, Olendzki BC, Jackson E, Stanek EJ 3rd, Li W, Pagoto SL, Hafner AR, Ockene IS. Association between dietary fiber and serum C-reactive protein. Am J Clin Nutr. 2006; 83(4): 760-766.

Madigan C, Ryan M, Owens D, Collins P, Tomkin GH. Dietary unsaturated fatty acids in type 2 diabetes: higher levels of postprandial lipoprotein on a linoleic acid-rich sunflower oil diet compared with an oleic acid-rich olive oil diet. Diabetes Care. 2000; 23(10): 1472-1477.

Magee MF, Bhatt BA. Management of decompensated diabetes. Diabetic ketoacidosis and hyperglycemic hyperosmolar syndrome. Crit Care Clin. 2001; 17:75-106.

Mamo JCL, Smith D, Yu KCW, Kawaguchi A, Harada-Shiba M, Yamamura T, Yamamoto A. accumulation of chylomicron in homozyhous subjects with familial hypercholesterolaemia. European Journal of Clinical Investigation. 1998; 28: 379-384

Mamo JCL. Atherosclerosis as a post-prandial disease. Endocrinology and metabolism. 1995; 2:229-244.

Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T,Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F. 2013

ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens. 2013; 31(7): 1281-1357.

Manco M, Calvani M, Mingrone G. Effects of dietary fatty acids on insulin sensitivity and secretion. Diabetes Obes Metab. 2004; 6(6): 402-413.

Manco M, Mingrone G, Greco AV, Capristo E, Gniuli D, De Gaetano A, Gasbarrini G. Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides. Metabolism. 2000; 49(2): 220-224.

Mantzoros CS. Obesity and Diabetes. Humana Press Inc. Totowa, New Jersey, (2006); p 71.

Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. J Intern Med. 2006; 259:437-446.

Marcovina S, Packard CJ. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. J Intern Med. 2006; 259(5): 437-446.

Meigs JB, Nathan DM, D'Agostino RB Sr, Wilson PW. Fasting and post-challenge glycemia and cardiovascular disease risk: the Framingham Offspring Study. Diabetes Care. 2002; 25: 1845-1850.

Meigs JB, O'donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PW. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: The Framingham Offspring Study. Diabetes. 2006; 55(2):530-537.

Mero N, Malmström R, Steiner G, Taskinen MR, Syvänne M. Postprandial metabolism of apolipoprotein B-48- and B-100-containing particles in type 2 diabetes mellitus: relations to angiographically verified severity of coronary artery disease. Atherosclerosis. 2000 ;150(1): 167-177.

Mitrou PN, Kipnis V, Thiébaut AC, Reedy J, Subar AF, Wirfält E, Flood A, Mouw T, Hollenbeck AR, Leitzmann MF, Schatzkin A. Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Arch Intern Med. 2007; 167(22): 2461-2468.

Mohsen Ibrahim M. Subcutaneous and visceral adipose tissue: structural and functional differences. Obesity Reviews. 2010; 11 (1): 11–18.

Monnier L, Colette C, Dunseath GJ, Owens DR. The loss of postprandial glycemic control precedes stepwise deterioration of fasting with worsening diabetes. Diabetes Care. 2007; 30:263–269.

Monnier L, Colette C. Target for Glycemic Control. Diabetes Care. 2009; 32(Suppl 2): S199–S204.

Monnier L, Colette C, Owens D. Postprandial and basal glucose in type 2 diabetes: assessment and respective impacts. Diabetes Technol Ther. 2011; 13 Suppl 1: S25–32.

Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. Diabetes. 2000; 49:883–8.

Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet–induced obesity. Nature Med. 2004; 10, 739–743.

Mosekilde L. Vitamin D and the elderly. Clin Endocrinol (Oxf). 2005; 62(3): 265–281.

Movahed MR, Sattur S, Hashemzadeh M. Independent association between type 2 diabetes mellitus and hypertension over a period of 10 years in a large inpatient population. Clin Exp Hypertens. 2010; 32 (3): 198–201.

Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA. 1999; 282(16): 1523–1529.

Nadler JL. A new dietary approach to reduce the risk of type 2 diabetes? Diabetes Care. 2004; 27(1): 270–271.

Natarajan S, Glick H, Criqui M, Horowitz D, Lipsitz SR, Kinosian B. Cholesterol measures to identify and treat individuals at risk for coronary heart disease. Am J Prev Med. 2003; 25(1): 50–57.

Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical Management of Hyperglycemia in Type 2 Diabetes: A Consensus Algorithm

for the Initiation and Adjustment of Therapy: A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009; 32: 193-203.

NCEP (National Cholesterol Education Program) National Institutes of Health, National Heart, Lung, and Blood Institute. Third Report of the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Final report. Circulation. 2002;106(25): 3143-3421.

NCEP (National Cholesterol Education Program): Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The NCEP Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001; 285(19): 2486-2497.

NIH (National Institutes of Health). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults -The Evidence Report. Obes Res 1998; 6 (Suppl 2):51S-209S.

Nikooyeh B, Neyestani TR, Farvid M, Alavi-Majd H, Houshiarrad A, Kalayi A, Shariatzadeh N, Gharavi A, Heravifard S, Tayebinejad N, Salekzamani S,Zahedirad M. Daily consumption of vitamin D- or vitamin D + calcium-fortified yogurt drink improved glycemic control in patients with type 2 diabetes: a randomized clinical trial. Am J Clin Nutr. 2011; 93(4): 764-771.

Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006; 295(13): 1556-1565.

Nogaroto V, Rodrigues MR, Vicari MR, Almeida MC, Milléo FQ, Santos FA, Artoni RF. High Postprandial Triglycerides Serum Levels: Is Obesity a Good Predictor?. An Acad Bras Cienc. 2015;0:0.

Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007; 298(3): 299-308.

Norris SL, Lee N, Thakurta S, Chan BKS. Exenatide efficacy and safety: a systematic review. Diabetic Medicine. 2009; 26: 837–846.

Norris SL, Zhang X, Avenell A, Gregg E, Schmid CH, Lau J. Pharmacotherapy for weight loss in adults with type 2 diabetes mellitus. Cochrane Database Syst Rev. 2005; (2):CD004095.

NRC (National Research Council) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, DC: The National Academies Press, 2005.

Ntyintyane LM, Panz VR, Raal FJ, Gill GV. Postprandial lipaemia, metabolic syndrome and LDL particle size in urbanised South African blacks with and without coronary artery disease. QJM. 2008; 101(2): 111–119.

NutriSurvey program 2007 for windows, SEAMEO-TROPMED RCCN-University of Indonesia (NutriSurvey, 2007).

Nye C, Kim J, Kalhan SC, Hanson RW. Reassessing triglyceride synthesis in adipose tissue. Trends in Endocrinology and Metabolism. 2008; 19(10).

O'Connell BS. Select Vitamins and Minerals in the Management of Diabetes. Diabetes Spectrum August. 2001; 14(3): 133–148.

Olshansky J, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med. 2005; 352(11): 1135–1137.

Otero LM, Zanetti ML, Teixeira CR. Sociodemographic and clinical characteristics of a diabetic population at a primary level health care center. Rev Lat Am Enfermagem. 2007; 15 (Spec No): 768–773.

Otten JJ, Hellwig JP, Meyers LD. Dietary Reference Intakes DRI The Essential Guide to Nutrient Requirements. Washington, DC: The National Academies Press, 2006.

Özkaya TG. The Role of Physical Exercise on Lipid Peroxidation in Diabetic Complications, Lipid Peroxidation, Dr. Angel Catala (Ed.) InTech, 2012.

Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal Muscle Triglyceride Levels Are Inversely Related to Insulin Action. Diabetes. 1997 ; 46(6): 983-988.

Paniagua JA, Gallego de la Sacristana A, Romero I, Vidal-Puig A, Latre JM, Sanchez E, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Monounsaturated fat-rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. Diabetes Care. 2007; 30(7): 1717-1723.

Parati G, Lombardi C, Narkiewicz K. Sleep apnea: epidemiology, pathophysiology, and relation to cardiovascular risk. Am J Physiol Regul Integr Comp Physiol. 2007; 293: R1671-R1683.

Parikh P, Mani U, Iyer U. Abdominal Adiposity and Metabolic Control in Patients With Type 2 Diabetes Mellitus. Iint J Diab Dev Countries. 2002; 22: 28-34.

Park SH, Lee KS, Park HY. Dietary carbohydrate intake is associated with cardiovascular disease risk in Korean: analysis of the third Korea National Health and Nutrition Examination Survey (KNHANES III). Int J Cardiol. 2010; 139(3): 234-240.

Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res. 2003; 44(3): 455-463.

Parks E. Recent findings in the study of postprandial lipemia. Current Atherosclerosis Reports. 2001; 3: 462-470.

Paulev PE, Zubieta-Calleja G. Textbook in Medical Physiology And Pathophysiology Essentials and clinical problems. Copenhagen Medical Publishers, 2012.

Pedrini MT, Niederwanger A, Kranebitter M, Tautermann C, Ciardi C, Tatarczyk T, Patsch JR. Postprandial lipaemia induces an acute decrease of insulin sensitivity in healthy men independently of plasma NEFA levels. Diabetologia. 2006; 49(7): 1612-1618.

Pena M, Bacallao J. Obesity and Poverty: A New Public Health Challenge. Pan American Health Organization (PAHO). Washington, DC, (2000).

Pereira T. Dyslipidemia and Cardiovascular Risk: Lipid Ratios as Risk Factors for Cardiovascular Disease, Dyslipidemia - From Prevention to Treatment, Editions Roya Kelishadi, (2012).

Pérez-Jiménez F, López-Miranda J, Pinillos MD, Gómez P, Paz-Rojas E, Montilla P, Marín C, Velasco MJ, Blanco-Molina A, Jiménez Perepérez JA, Ordovás JM. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. Diabetologia. 2001; 44(11): 2038-2043.

Perez-Martinez P, Delgado-Lista J, Perez-Jimenez F, Lopez-Miranda J. Update on genetics of postprandial lipemia. Atherosclerosis Supplements. Atheroscler Suppl. 2010; 11(1): 39-43.

Perez-Martinez P, Yiannakouris N, Lopez-Miranda J, Arnett D, Tsai M, Galan E, Straka R, Delgado-Lista J, Province M, Ruano J, Borecki I, Hixson J, Garcia-Bailo B, Perez-Jimenez F, Ordovas JM. Postprandial triacylglycerol metabolism is modified by the presence of genetic variation at the perilipin (PLIN) locus in 2 white populations. Am J Clin Nutr 2008; 87: 744-52.

Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F, Ordovas JM. Influence of genetic factors in the modulation of postprandial lipemia. Atheroscler Suppl. 2008; 9(2): 49-55.

Phillips CM, Kesse-Guyot E, McManus R, Hercberg S, Lairon D, Planells R, Roche HM. High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. J Nutr. 2012; 142(5): 824-831.

Phillips WT, Schwartz JG, McMahan CA. Reduced postprandial blood glucose levels in recently diagnosed non-insulin-dependent diabetics secondary to pharmacologically induced delayed gastric emptying. Dig Dis Sci. 1993; 38: 51-58.

Popkin BM. The nutrition transition and obesity in the developing world. Journal of Nutrition. 2001; 131(3): 871S-3S.

Pownall HJ, Ballantyne CM, Kimball KT, Simpson SL, Yeshurun D, Gotto AM Jr. Effect of moderate alcohol consumption on hypertriglyceridemia: A study in the fasting state. Arch Intern Med. 1999; 159: 981-987.

Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. Diabetologia. 2001; 44(8): 929-45.

Prentice AM, Black A, Goldberg G. Diabetes and energy intake. Am J Clin Nutr.1993; 57: 596 -598.

Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest. 2006; 116(7): 1802-1812.

Qiao Q, Hu G, Tuomilehto J, Nakagami T, Balkau B, Borch-Johnsen K, Ramachandran A, Mohan V, Iyer SR, Tominaga M, Kiyohara Y, Kato I, Okubo K, Nagai M, Shibazaki S, Yang Z, Tong Z, Fan Q, Wang B, Chew SK, Tan BY, Heng D, Emmanuel S, Tajima N, Iwamoto Y, Snehalatha C, Vijay V, Kapur A, Dong Y, Nan H, Gao W, Shi H, Fu F; DECODE Study Group. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes Care. 2003; 26(6): 1770-1780.

Qin LQ, Xun P, Bujnowski D, Daviglus ML, Van Horn L, Stamler J, He K. Higher branched-chain amino acid intake is associated with a lower prevalence of being overweight or obese in middle-aged East Asian and Western adults. J Nutr. 2011; 141(2): 249-254.

Rajkumar R, Preedy VR, Patel VB. Branched Chain Amino Acids in Clinical Nutrition. Springer New York, (2015); p. 287.

Ramlo-Halsted BA, Edelman SV. The natural history of type 2 diabetes. Implications for clinical practice. Prim Care. 1999; 26(4): 771-789.

Rave K, Sidharta PN, Dingemanse J, Heinemann L, Roggen K. First-Phase Insulin Secretion Has Limited Impact on Postprandial Glycemia in Subjects with Type 2 Diabetes: Correlations Between Hyperglycemic Glucose Clamp and Meal Test. Diabetes Technology & Therapeutics.2010; 12(2):117-123.

Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann N Y Acad Sci. 2002; 967: 363-378.

Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. Annu Rev Nutr. 2005; 25: 391-406.

Relas H, Gylling H, Miettinen TA. Effect of stanol ester on postabsorptive squalene and retinyl palmitate. Metabolism. 2000; 49(4): 473-478.

Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation. 2003; 107(3):391-397.

Risérus U, Arnlöv J, Berglund L. Long-term predictors of insulin resistance: role of lifestyle and metabolic factors in middle-aged men. Diabetes Care. 2007; 30(11): 2928-2933.

Rodriguez MM, Guerrero JFR Importancia del apoyo familiar en el control de la glicemia. Salud Pública Méx. 1997; 39(1): 44-47.

Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, Connolly V, King H. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes Care. 2005; 28(9): 2130-2135.

Rojas LB, Gomes MB. Metformin: an old but still the best treatment for type 2 diabetes. Diabetol Metab Syndr. 2013; 5(1): 6.

Rolls BJ. The Supersizing of America: portion size and the obesity epidemic. Nutr Today. 2003; 38(2):42-53.

Romero-Corral A, Sierra-Johnson J, Lopez-Jimenez F, Thomas RJ, Singh P, Hoffmann M, Okcay A, Korinek J, Wolk R, Somers VK. Relationships between leptin and C-reactive protein with cardiovascular disease in the adult general population. Nat Clin Pract. 2008; 5:418-425.

Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol. 2000;16:145-171.

Ross R. Atherosclerosis-an inflammatory disease. N Engl J Med. 1999; 340(2):115-126.

Rungby J. Zinc, zinc transporters and diabetes. Diabetologia. 2010; 53(8):1549-1551.

Rydén L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer MJ, Cosentino F, Jönsson B, Laakso M, Malmberg K, Priori S, Ostergren J, Tuomilehto J, Thrainsdottir

I, Vanhorebeek I, Stramba-Badiale M, Lindgren P, Qiao Q, Priori SG, Blanc JJ, Budaj A, Camm J, Dean V, Deckers J, Dickstein K, Lekakis J,McGregor K, Metra M, Morais J, Osterspey A, Tamargo J, Zamorano JL, Deckers JW, Bertrand M, Charbonnel B, Erdmann E, Ferrannini E, Flyvbjerg A, Gohlke H, Juanatey JR, Graham I, Monteiro PF, Parhofer K, Pyörälä K, Raz I, Schernthaner G, Volpe M, Wood D. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J. 2007; 28(1): 88-136.

Rzonca SO, Suva LJ, Gaddy D, Montague DC, Lecka-Czernik B. Bone is a target for the antidiabetic compound rosiglitazone. Endocrinology. 2004; 145: 401-406.

Salazar MR, Carbajal HA, Espeche WG, Aizpurúa M, Maciel PM, Reaven GM. Identification of cardiometabolic risk: visceral adiposity index versus triglyceride/HDL cholesterol ratio. Am J Med. 2014; 127(2): 152-157.

Sallé A, Ryan M, Ritz P. Underreporting of Food Intake in Obese Diabetic and Nondiabetic Patients. Diabetes Care. 2006; 29(12): 2726-2727.

Salpeter S, Greyber E, Pasternak G, Salpeter E. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 2006;1: CD002967.

Samaras K, Campbell LV. Increasing incidence of type 2 diabetes in the third millennium: is abdominal fat the central issue? Diabetes Care. 2000; 23:441-442.

Sampson MJ, Gopaul N, Davies IR, Hughes DA, Carrier MJ. Plasma F2 isoprostanes: direct evidence of increased free radical damage during acute hyper- glycemia in type 2 diabetes. Diabetes Care. 2002; 25: 537-541.

Sandoval D, Cota D, Seeley RJ. The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation. Annu Rev Physiol. 2008; 70: 513-535.

Schillinger D, Grumbach K, Piette J, Wang F, Osmond D, Daher C, Palacios J, Sullivan GD, Bindman AB. Association of health literacy with diabetes outcomes. JAMA. 2002; 288(4): 475-482.

Schlienger JL, Luca F, Griffon C. Déficit en vitamine D et risque de diabète. Médecine des Maladies Métaboliques. 2010 ; 4(5) : 558-562.

Schmidt PT, Na¨slund E, Gryba¨ ck P, Jacobsson H, Holst JJ, Hilsted L, Hellstro¨m PM. A Role for Pancreatic Polypeptide in the Regulation of Gastric Emptying and Short-Term Metabolic Control. J Clin Endocrinol Metab. 2005; 90(9): 5241-5246.

Scobie IN. Atlas of Diabetes Mellutis 3rd Edition. Taylor & Francis, (2007); p 120.

Scott TL, Gazmararian JA, Williams MV, Baker DW. Health literacy and preventive health care use among Medicare enrollees in a managed care organization. Med Care. 2002; 40(5): 395-404.

Seale P, Lazar AM. Brown Fat in Humans: Turning up the Heat on Obesity. Diabetes. 2009; 58.

Sharrett AR, Heiss G, Chambless LE, Boerwinkle E, Coady SA, Folsom AR, Patsch W. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: The Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2001; 21: 275-281.

Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010; 87(1): 4-14.

Shen J, Arnett DK,Pérez-Martínez P,Parnell LD,Lai CQ,Peacock JM,Hixson JE,Tsai MY,Straka RJ,Hopkins PN,Ordovás JM. The effect of IL6-174C/G polymorphism on postprandial triglyceride metabolism in the GOLDN study. Journal of Lipid Research. 2008; 49(8):1839-1845.

Shilpasree AS, Sahukar S, Murthy J, Kumar K. A Study of Serum Apolipoprotein A1, Apolipoprotein B and Lipid Profile in Stroke. J Clin Diagn Res. 2013; 7(7): 1303-1306.

Sierra-Johnson J, Fisher RM, Romero-Corral A, Somers VK, Lopez-Jimenez F, Ohrvik J, Walldius G, Hellenius ML, Hamsten A. Concentration of apolipoprotein B is comparable with the apolipoprotein B/apolipoprotein A-I ratio and better than routine clinical lipid measurements in predicting coronary heart disease mortality: findings from a multi-ethnic US population. Eur Heart J. 2009; 30(6): 710-717.

Silvestre R, Kraemer WJ, Quann EE, Seip RL, Maresh CM, Vingren JL, Hatfield DL, Volek JS. Effects of exercise at different times on postprandial lipemia and endothelial function. Med Sci Sports Exerc. 2008; 40: 264–274.

Simcox JA, McClain DA. Iron and Diabetes Risk. Cell Metab. 2013; 17(3): 329–341.

Singla P, Bardoloi A, Parkash AA. Metabolic effects of obesity: A review. World J Diabetes. 2010; 1(3): 76–88.

Slentz CA, Duscha BD, Johnson JL, Ketchum K, Aiken LB, Samsa GP, Houmard JA, Bales CW, Kraus WE. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE – a randomized controlled study. Arch Intern Med. 2004; 164:31–9.

Smit LA, Mozaffarian D, Willett W. Review of fat and fatty acid requirements and criteria for developing dietary guidelines. Ann Nutr Metab. 2009 ;55(1–3): 44–55.

Smith BK, Sun GY, Donahue OM, Thomas TR. Exercise plus n-3 fatty acids: Additive effect on postprandial lipemia. Metabolism. 2004; 53:1365–1371.

Song X, Tabák AG, Zethelius B, Yudkin JS, Söderberg S, Laatikainen T, Stehouwer CD, Dankner R, Jousilahti P, Onat A, Nilsson PM, Satman I, Vaccaro O, Tuomilehto J, Qiao Q for the DECODE Study Group. Obesity attenuates gender differences in cardiovascular mortality. Cardiovascular Diabetology. 2014; 13:144.

Song Y, Cook NR, Albert CM, Van Denburgh M, Manson JE. Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr. 2009; 90: 253–254.

Sorkin JD, Muller DC, Fleg JL, Andres R. The relation of fasting and 2-h postchallenge plasma glucose concentrations to mortality: data from the Baltimore Longitudinal Study of Aging with a critical review of the literature. Diabetes Care. 2005; 28(11): 2626–2632.

Sowers JR, Epstein M, Frohlich ED. Diabetes, hypertension, and cardiovascular disease: an update. Hypertension. 2001; 37(4): 1053–1059.

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Näslund E, Britton T, Concha H, Hassan M, Rydén M,Frisén J, Arner P. Dynamics of fat cell turnover in humans. Nature. 2008; 453(7196):783-787.

SPSS Statistical Package for the Social Sciences, IBM Corporation; Chicago, IL August 2011) for Windows (SPSS, 2011).

Stampfer MJ, Krauss RM, Ma J, Blanche PJ, Holl LG, Sacks FM, Hennekens CH. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA. 1996; 276(11): 882-888.

Steptoe A, Brydon L. Associations Between Acute Lipid Stress Responses and Fasting Lipid Levels 3 Years Later. Health Psychology. 2005; 24(6): 601- 607.

Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, Gracely EJ, Samaha FF. The effects of lowcarbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. Ann Intern Med. 2004; 140: 778-785.

Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia. 2002; 45(3): 369-377.

Swinburn BA, Caterson I, Seidell JC, James WPT. Diet, nutrition and the prevention of excess weight gain and Obesity, Public Health Nutrition. 2004; 7(1A): 123-146.

Szalai C, Keszei M, Duba J, Prohászka Z, Kozma GT, Császár A, Balogh S, Almássy Z, Fust G, Czinner A. Polymorphism in the promoter region of the apolipoprotein A5 gene is associated with an increased susceptibility for coronary artery disease. Atherosclerosis. 2004; 173(1): 109-14.

Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol. 1999; 276(5 Pt 1): E977-E989.

Tamang HK, Timilsina U, Singh KP, Shrestha S, Raman RK, Panta P, Karna P, Khadka L, Dahal C. Apo B/Apo A-I Ratio is Statistically A Better Predictor of Cardiovascular

Disease (CVD) than Conventional Lipid Profile: A Study from Kathmandu Valley, Nepal. J Clin Diagn Res. 2014; 8(2): 34-36.

Taskinen MR, Barter PJ, Ehnholm C, Sullivan DR, Mann K, Simes J, Best JD, Hamwood S, Keech AC; FIELD study investigators. Ability of traditional lipid ratios and apolipoprotein ratios to predict cardiovascular risk in people with type 2 diabetes. Diabetologia. 2010; 53(9): 1846-1855.

Teno S, Uto Y, Nagashima H, Endoh Y, Iwamoto Y, Omori Y, Takizawa T. Association of postprandial hypertriglyceridemia and carotid intima-media thickness in patients with type 2 diabetes. Diabetes Care. 2000; 23(9):1401-1406.

Tertov VV, Kalenich OS, Orekhov AN. Lipid-laden white blood cells in the circulation of patients with coronary heart disease. Exp Mol Pathol. 1992; 57(1): 22-28.

TFRNS (Task Force to Revise the National Standards) The American Diabetes Association. National standards for diabetes self-management education programs. Diabetes Educ. 1995; 21: 189-193.

Thomsen C, Rasmussen O, Lousen T, Holst JJ, Fenselau S, Schrezenmeir J, Hermansen K. Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. Am J Clin Nutr. 1999; 69(6): 1135-1143.

Tietz NW. Fundamentals of clinical chemistry, 3rd ed. Saunders Philadelphia, WB; 1987.

Torgerson JS, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care. 2004; 27: 155-161.

Tounian P. L'obésité de l'enfant. Edition John Libbey-Eurotext, (2006).

Trayhurn P. Adipocyte biology. Obesity Reviews. 2007; 8 (Suppl. 1): 41-44.

Turchin A, Matheny ME, Shubina M, Scanlon JV, Greenwood B, Pendergrass ML. Hypoglycemia and clinical outcomes in patients with diabetes hospitalized in the general ward. Diabetes Care. 2009; 32, 1153-1157.

UKPDS (UK Prospective Diabetes Study) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998 ; 12; 352(9131): 837-853.

Umpierre D, Ribeiro PA, Kramer CK, Leitão CB, Zucatti AT, Azevedo MJ, Gross JL, Ribeiro JP, Schaan BD. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. JAMA. 2011; 305(17): 1790-1799.

Unger RH. How obesity causes diabetes in Zucker diabetic fatty rats. Trends Endocrinol Metab. 1997; 8(7): 276-282.

Valcour A, Blocki F, Hawkins DM, Rao SD. Effects of age and serum 25-OH-vitamin D on serum parathyroid hormone levels. J Clin Endocrinol Metab. 2012; 97(11): 3989-3995.

van der Steeg WA, Boekholdt SM, Stein EA, El-Harchaoui K, Stroes ES, Sandhu MS, Wareham NJ, Jukema JW, Luben R, Zwinderman AH, Kastelein JJ, Khaw KT. Role of the apolipoprotein B-apolipoprotein A-I ratio in cardiovascular risk assessment: a case-control analysis in EPIC-Norfolk. Ann Intern Med. 2007; 146(9): 640-648.

Van Haeften TW, Pimenta W, Mitrakou A, Korytkowski M, Jenssen T, Yki-Jarvinen H, Gerich JE. Relative contributions of beta-cell function and tissue insulin sensitivity to fasting and postglucose-load glycemia. Metabolism. 2000; 49: 1318-1325.

Van Horn L, McCoin M, Kris-Etherton PM, Burke F, Carson JA, Champagne CM, Karmally W, Sikand G.. The evidence for dietary prevention and treatment of cardiovascular disease. J Am Diet Assoc. 2008; 108: 287-331.

Van Oostrom AJ, Plokker HW, van Asbeck BS, Rabelink TJ, van Kessel KP, Jansen EH, Stehouwer CD, Cabezas MC. Effects of rosuvastatin on postprandial leukocytes in mildly hyperlipidemic patients with premature coronary sclerosis. Atherosclerosis. 2006; 185(2): 331-339.

Veghari G, Sedaghat M, Joshaghani H, Hoseini SA, Niknezad F, Angizeh A, Tazik E, Moharloei P. Association between socio-demographic factors and diabetes mellitus in

the north of Iran: A population-based study. International Journal of Diabetes Mellitus 2. 2010; 2: 154-157.

Verseyden C, Meijssen S, Castro Cabezas M. Effects of atorvastatin on fasting plasma and marginated apolipoproteins B48 and B100 in large, triglyceride-rich lipoproteins in familial combined hyperlipidemia. J Clin Endocrinol Metab. 2004; 89: 5021-5029.

Vinik A. The protein kinase C-β inhibitor, ruboxistaurin, for the treatment of diabetic microvascular complications. Expert Opin Investig Drugs. 2005; 14: 1547-1559.

Visockienė Ž, Broom JI, Speakman JR, Johnstone AM. Dietary Intake and Self-Reporting Relation to Eating Behaviour in Obese and Type 2 Diabetes Patients. Baltic Endocrinology. 2006; 2(1): 24-30.

von Bibra H, Paulus WJ, St John Sutton M, Leclerque C, Schuster T, Schumm-Draeger PM. Quantification of diastolic dysfunction via the age dependence of diastolic function - Impact of insulin resistance with and without type 2 diabetes. Int J Cardiol. 2014; 182: 368-374.

Wägner AM, Pérez A, Sánchez-Quesada JL, Ordóñez-Llanos J. Triglyceride-to-HDL cholesterol ratio in the dyslipidemic classification of type 2 diabetes. Diabetes Care. 2005; 28(7): 1798-800.

Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet. 2001; 358(9298): 2026-2033.

Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. Am J Clin Nutr. 2005; 81(3): 555-563.

Wasan KM, Looije NA. Emerging Pharmacological Approaches to the Treatment of Obesity. J Pharm Pharmaceut Sci 8. 2005; (2):259-271.

Watanabe N, Taniguchi T, Taketoh H, Kitagawa Y, Namura H, Yoneda N, Kurimoto Y, Yamada S, Ishikawa Y. Elevated remnant-like lipoprotein particles in impaired glucose tolerance and type 2 diabetic patients. Diabetes Care. 1999; 22(1): 152-156.

Watkins PJ. ABC OF Diabetes. Fifth Edition. BMJ Publishing Group Ltd. London, (2003); p. 95.

Watkins PJ. Retinopathy. BMJ. 2003; 326: 924-926.

Watts GF, Mamo JCL, Redgrave TG. Postprandial dyslipidaemia in a nutshell: food for thought. Australian and New Zealand Journal of Medicine. 1998; 28: 816-823.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112(12): 1796-1808.

Weiss R, Dufour S, Taksali SE, Tamborlane WV, Petersen KF, Bonadonna RC, Boselli L, Barbetta G, Allen K, Rife F, Savoye M, Dziura J, Sherwin R, Shulman GI, Caprio S. Prediabetes in obese youth: a syndrome of impaired glucose tolerance, severe insulin resistance, and altered myocellular and abdominal fat partitioning. Lancet. 2003; 362(9388): 951-957.

WHO (World Health Organisation) Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Geneva, (1999).

WHO (World Health Organization) Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. Report of a WHO/IDF Consultation. Geneva. (2006).

WHO (World Health Organization) Global health risks: mortality and burden of disease attributable to selected major risks. Geneva, (2009).

WHO (World Health Organization) Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. WHO Technical Report Series No. 894. Geneva, (2000).

WHO (World Health Organization) World health statistics 2012. World Health Organization. Geneva, (2012).

WHO/FAO (World Health Organization/Food and Agriculture Organization) Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation, N° 916. Geneva, 2003.

Wild SH, Byrne CD. Risk factors for diabetes and coronary heart disease. BMJ. 2006; 333 (11).

Wolever TM, Bolgnesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. J Nutr. 1996; 126: 2798-2806.

Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA,Nordestgaard BG, Ridker P, Salomaa V, Stevens J, Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J. The Emerging Risk Factor Collaboration. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet. 2011; 377:1085-1095.

Wright SM, Aronne LJ. Causes of obesity. Abdom Imaging. 2012; 37(5):730-2.

Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, Donker AJ, Stehouwer CD. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J Intern Med. 2003; 254(5): 455-463.

Yamagishi S I, Nakamura K, Matsui T, Ueda SI, Imaizumi T. Role of postprandial hyperglycaemia in cardiovascular disease in diabetes. Int J Clin Pract. 2007; 61 (1): 83-87.

Yan Z, Liu Y, Huang H. Association of glycosylated hemoglobin level with lipid ratio and individual lipids in type 2 diabetic patients. Asian Pac J Trop Med. 2012; 5(6): 469-471.

Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364(9438): 937-952.

Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallelgroup study. Lancet. 2002; 359: 824-830.

Zhang JQ, Smith B, Langdon MM, Messimer HL, Sun GY, Cox RH, James-Kracke M, Thomas TR. Changes in LPLa and reverse cholesterol transport variables during 24-h postexercise period. Am J Physiol Endocrinol Metab. 2002; 283: E267-E274.

Zhao W, Gong W, Wu N, Li Y, Ye K, Lu B, Zhang Z, Qu S, Li Y, Yang Y, Hu R. Association of lipid profiles and the ratios with arterial stiffness in middle-aged and elderly Chinese. Lipids Health Dis. 2014; 13: 37.

Zhou G, Myers R, Li Y,Chen Y,Shen X, Fenyk-Melody J, Wu M,Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of Clinical Investigation. 2001; 118 (8): 1167-1174.

Zilversmit DB. Atherogenesis: A postprandial phenomenon. Circulation. 1979; 60 (3): 473-485.

Annex 1

SPINREACT

CE GLUCOSE -LQ

Glucose

GOD-POD. Liquid

Quantitative determination of glucose
IVD
Store at 2-8ºC

PRINCIPLE OF THE METHOD

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H_2O_2), is detected by a chromogenic oxygen acceptor, phenol, 4 – aminophenazone (4-AP) in the presence of peroxidase (POD):

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample[1,2].

CLINICAL SIGNIFICANCE

Glucose is a major source of energy for most cells of the body; insulin facilitates glucose entry into the cells.
Diabetes is a disease manifested by hyperglycemia; patients with diabetes demonstrate an inability to produce insulin[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R	TRIS pH 7.4	92 mmol/L
	Phenol	0.3 mmol/L
	Glucose oxidase (GOD)	15000 U/L
	Peroxidase (POD)	1000 U/L
	4 – Aminophenazone (4-AP)	2.6 mmol/L

PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.

SIGNS OF REAGENT DETERIORATION:

- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm $\geq$ 0.32.

ADDITIONAL EQUIPMENT

- MINDRAY BS-120 / BS-200E Autoanalyzer.
- General laboratory equipment.

SAMPLES

Serum or plasma, free of hemolysis[1]:
Serum should be removed from the clot as quickly as possible.
Stability of the sample: Glucose in serum or plasma is stable at 2-8º for 3 days.

REFERENCE VALUES[1]

Serum or plasma:
60 – 110 mg/dL $\cong$ 3.33 – 6.10 mmol/L
These values are for orientation purpose; each laboratory should establish its own reference range.

MINDRAY BS-120 / BS-200E APPLICATION

PARAMETERS			
Test	GLU / GLU	R1	300 / 300
Nº	**	R2	*
Full Name	GLU / GLU	Sample volume	3 / 3
Standard Nº		R1 Blank	
Reac. Type	Endpoint / Endpoint	Mixed Rgt Blank	
Pri. Wavelength	510 / 505	Linearity Range	0 mg/dL 500 mg/dL
Sec. Wavelength		Linearity Limit	*
Direction	Increase / Increase	Substrate Limit	*
Reac. Time	1_33 / 0_33	Factor	*
Incuba. Time		Prozone check	*
Units	mg/dL / mg/dL	q1	q2
Precision	Interger / Interger	q3	q4
		PC	Abs

CALIBRATION (Cal + Rgt Blk)	
Rule	One-point Linear / Two-point Linear
Sensitivity	1 / 1
Replicates	2 / 2
Interval (days)	0 / 0
Difference Limit	
SD	
Blank Response	
Error Limit	
Correlation Coefficient	

*Blank parameter must be performed in order to get good results in CALIB screen from main menu. The blank calibration is stable until **35 days.** After this period the blank parameter must be performed again in order to validate the calibration.*

QUALITY CONTROL

Control sera and calibrators are recommended to monitor the performance of assay procedures: SPINTROL H Calibrator, SPINTROL H Normal and Pathologic (Ref. 1002011, 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

1. Kaplan L.A. Glucose. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1032-1036.
2. Trinder P. Ann Clin Biochem 1969; 6 24-33.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: MI41011	Cont.	R: 6 x 30 mL

SPINREACT,S.A/S.A.U Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com

CHOLESTEROL -LQ

Cholesterol

CHOD-POD. Liquid

Quantitative determination of cholesterol
IVD
Store at 2-8ºC

PRINCIPLE OF THE METHOD

The cholesterol present in the sample originates a coloured complex, according to the following reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample[1,2].

CLINICAL SIGNIFICANCE

Cholesterol is a fat-like substance called a lipid that is found in all body cells. The liver makes all of the cholesterol the body needs to form cell membranes and to make certain hormones.
The determination of serum cholesterol is one of the important tools in the diagnosis an classification of lipemia.
High blood cholesterol is one of the major risk factors for heart disease[5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R	PIPES pH 6.9	90 mmol/L
	Phenol	26 mmol/L
	Cholesterol esterase (CHE)	1000 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	Peroxidase (POD)	650 U/L
	4 – Aminophenazone (4-AP)	0.4 mmol/L

PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm $\geq$ 0.26.

ADDITIONAL EQUIPMENT

- MINDRAY BS-120 / BS-200E Autoanalyzer.
- General laboratory equipment.

SAMPLES

Serum or plasma[1,2]: Stability of the sample 7 days at 2-8ºC or freezing at –20ºC will keep samples stable for 3 months.

REFERENCE VALUES

Risk evaluation[5,6]:

Less than 200 mg/dL	Normal
200-239 mg/dL	Borderline
240 mg/dL and above	High

These values are for orientation purpose; each laboratory should establish its own reference range.

MINDRAY BS-120 / BS-200E APPLICATION

PARAMETERS			
Test	CHOL / CHOL	R1	300 /300
Nº	**	R2	*
Full Name	CHOL / CHOL	Sample volume	3 / 3
Standard Nº		R1 Blank	
Reac. Type	Endpoint / Endpoint	Mixed Rgt Blank	
Pri. Wavelength	510 / 505	Linearity Range	0 mg/dL 600 mg/dL
Sec. Wavelength		Linearity Limit	*
Direction	Increase / Increase	Substrate Limit	*
Reac. Time	1_17 / 0_17	Factor	*
Incuba. Time		Prozone check	*
Units	mg/dL / mg/dL	q1	q2
Precision	Interger / Interger	q3	q4
		PC	Abs

CALIBRATION (Cal + Rgt Blk)	
Rule	One-point Linear / Two-point Linear
Sensitivity	1 / 1
Replicates	2 / 2
Interval (days)	0 / 0
Difference Limit	
SD	
Blank Response	
Error Limit	
Correlation Coefficient	

*Blank parameter must be performed in order to get good results in CALIB screen from main menu. The blank calibration is stable until **35 days.** After this period the blank parameter must be performed again in order to validate the calibration.*

QUALITY CONTROL

Control sera and calibrators are recommended to monitor the performance of assay procedures: SPINTROL H Calibrator, SPINTROL H Normal and Pathologic (Ref. 1002011, 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

NOTES

1. LCF (Lipid Clearing Factor) is integrated in the reagent.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

1. Naito H.K. Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1194-11206 and 437.
2. Meiattini F. et al. The 4-hydroxybenzoate/4-aminophenazone Chromogenic System. Clin Chem 1978; 24 (12): 2161-2165.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: MI41021	Cont.	R: 6 x 30 mL

SPINREACT,S.A./S.A.U Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com

Liquid Reagent Ready to use for:
SpinTech240
BIOLIS24i Premium
Prestige 24i
SAPPHIRE

CE HDLc -D

HDL Cholesterol

Direct. Enzymatic colorimetric

Quantitative determination of HDL cholesterol
IVD
Store at 2-8ºC

PRINCIPLE OF THE METHOD

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.
The assay takes place in two steps.
– 1º Elimination of lipoprotein no-HDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$
$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$
$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

– 2º Measurement of HDLc

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$
$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$
$$2\,H_2O_2 + \text{HDAOS} + \text{4-AA} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the HDLc concentration in the sample.

CLINICAL SIGNIFICANCE

HDL particles are high-density lipoproteins that transport cholesterol from the body tissues to the liver. Since HDL can remove cholesterol from the arteries and carry it back to the liver for their excretion, HDL is known as "good cholesterol" because high levels are thought to lower the risk of heart disease and coronary artery disease.
A low HDL cholesterol levels, is considered a greater heart disease risk[1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 6.6	100 mM
	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS)	0.7 mM
	Cholesterol Esterase	≥ 800 U/L
	Cholesterol oxidase	≥ 500 U/L
	Catalase	≥ 300 KU/L
	Ascorbic oxidase	≥ 3000 U/L
R 2	N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 7.0	100 mM
	4 – Aminoantipyrine (4-AP)	4 mM
	Peroxidase	≥ 3500 U/L

PREPARATION

- **R 1 and R 2**: Are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not freeze the reagents.
- **R 1 and R 2**: Once opened is stable 4 weeks at 2-8ºC.
Do not use reagents over the expiration date.
Signs of reagent deterioration:
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Autoanalyzer Spintech 240.
- General laboratory equipment.

SAMPLES

Serum or heparinized plasma, free of hemolysis[1]: Anticoagulants containing citrate should not be use.
Removed from the blood clot as soon as possible
Stability of the sample: 7 days at 2-8ºC .

REFERENCE VALUES[2]

	Men	Women
Low risk	> 50 mg/dL	> 60 mg/dL
Normal risk	35 – 50 mg/dL	45 – 60 mg/dL
High risk	< 35 mg/dL	< 45 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

APPLICATION SPINTECH 240

Item Name HDL

DATA INFORMATION
Units mg/dL
Decimals 1

ANALYSIS
Type END
W.Length 1 570
Method DIRECT

CORR
SLOPE INTER
1.000 x + 0

CALIBRATION
TYPE Linear

STANDARD
#1 * #4
#2 #5
#3 #6

NORMAL RANGE
LOW HIGH
SERUM MALE
FEMALE

Item Name HDL

ASPIRATION
KIND Single ✔ Double

VOLUME
SAMPLE 3 µL
REAGENT 1 225 µL
REAGENT 2 70 µL

Third Mix ✔ OFF ON
R1 Blank Water ✔ R1-B

MONITOR
0 LEVEL POINT 1
SPAN 3.000

DATA PROCESS
READ
START END
MAIN 50 51
SUB 28 29

FACTOR
Blank Correction 1.000

PROZONE CHECK
START END LIMIT (%)
FIRST
SECOND ✔ Low High
THIRD ✔ Low High

ABSORBANCE LIMIT
LOW -3.000
HIGH 3.000

ENDPOINT LIMIT 3
LINEAR CHECK (%)

*Blank parameter must be performed in order to get good results in CALIB screen from main menu. This parameter calibration is stable for more than **40 days.***

Conversion factor: mg/dL x 0.0259= mmol/L.

QUALITY CONTROL

Control sera and calibrators are recommended to monitor the performance of assay procedures: SPINTROL H Calibrator, SPINTROL H Normal and Pathologic (Ref. 1002011, 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

NOTES

The reagent 2 presents yellowish coloration due to the peroxidase, but it does not affect its functionality.

BIBLIOGRAPHY

1. Naito H K HDL Cholesterol. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1207-1213 and 437.
2. US National Cholesterol Education Program of the National Institutesof Health.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref:TK1001096	Cont.	R1: 10 x 24 mL, R2: 10 x 8 mL
Ref:TK1001097		R1: 2 x 24 mL, R2: 2 x 8 mL

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SPINREACT

CE LDLc -D

Cholesterol LDL

Enzymatic colorimetric. Liquid

Quantitative determination of LDL cholesterol
IVD
Store at 2-8ºC

PRINCIPLE OF THE METHOD

Direct determination of serum LDLc (low-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation steps.
The assay takes place in two steps.
–1º Elimination of lipoprotein no-LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

–2º Measurement of LDLc

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{TODS} + \text{4-AA} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the LDLc concentration in the sample.

CLINICAL SIGNIFICANCE

The LDLc particle is lipoproteins that transport cholesterol to the cells.
Often called “bad cholesterol” because high levels are risk factor for coronary heart disease and are associated with obesity, diabetes and nephrosis [1,5,6].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Enzymes	GOOD pH 7.0 (20ºC)	50 mmol/L
	Cholesterol esterase (CHE)	380 U/L
	Cholesterol oxidase (CHOD)	380 U/L
	Catalase	400 U/mL
	TODS	0.45 mmol/L
R 2 Enzymes	GOOD pH 7.0	50 mmol/L
	4 – Aminoantipyrine (4-AA)	1.00 mmol/L
	Peroxidase (POD)	1000 U/L

PREPARATION

R 1 and R 2: Are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use.
R 1 and R 2: Once opened is stable 4 weeks at 2-8ºC.
Signs of reagent deterioration:
- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- MINDRAY BS-120 / BS-200E Autoanalyzer.
- General laboratory equipment.

SAMPLES

Serum [1]: After sampling, the test should be performed without delay. Repeated freezing and thawing should be avoided.
Stability of the sample: 7 days at 2-8ºC .

REFERENCE VALUES[1,5,6]

Levels of the risk

Desirable	< 100 mg/dL
Medium	130-160 mg/dL
High	> 160 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

MINDRAY BS-120 / BS-200E APPLICATION

PARAMETERS				
Test	LDL / LDL	R1		225 / 225
Nº	**	R2		75 / 75
Full Name	LDL / LDL	Sample volume		3 / 3
Standard Nº		R1 Blank		
Reac. Type	Endpoint / Endpoint	Mixed Rgt Blank		
Pri. Wavelength	578 / 570 nm	Linearity Range	1.0 mg/dL	1000.0 mg/dL
Sec. Wavelength		Linearity Limit		*
Direction	Increa / Increa	Substrate Limit		*
Reac. Time	-1 _18 / -1 _18	Factor		*
Incuba. Time		Prozone check		*
Units	mg/dL / mg/dL	q1		q2
Precision	0.1 / 0.1	q3		q4
		PC		Abs

CALIBRATION (Cal + Rgt Blk)	
Rule	One-point Linear / Two-point Linear
Sensitivity	1 / 1
Replicates	2 / 2
Interval (days)	0 / 0
Difference Limit	
SD	
Blank Response	
Error Limit	
Correlation Coefficient	

*Blank parameter must be performed in order to get good results in CALIB screen from main menu. The blank calibration is stable until **35 days.** After this period the blank parameter must be performed again in order to validate the calibration.*

QUALITY CONTROL

Control sera and calibrators are recommended to monitor the performance of assay procedures: SPINTROL H Calibrator, SPINTROL H Normal and Pathologic (Ref. 1002011, 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

BIBLIOGRAPHY

1. Kaplan A et al. Lipoprotein. Clin Chem The C.V. Mosby Co. St Louis.
2. Okada M. et al. Low-density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Mad., 1998; 132, 195-201.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PACKAGING

Ref: MI41023	Cont.	R 1: 4 x 30 mL R 2: 2 x 20 mL

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SPINREACT

CE TRIGLYCERIDES

Triglycérides

GPO-POD. Enzymatique colorimétrique

Détermination quantitative de triglycérides
IVD

Conserver à 2-8ºC

PRINCIPE DE LA METHODE

Les triglycérides incubés avec de la lipoprotéinlipase (LPL) libèrent du glycérol et des acides gras libres. Le glycérol est phosphorilasé par du glycérophosphate déshydrogénase (GPO) et de l'ATP en présence de glycérol kinase (GK) pour produire du glycérol-3-phosphate (G3P) et de l'adénosine-5-di phosphate (ADP). Le G3P est alors transformé en dihydroxiacétone phosphate (DAP) et en peroxyde d'hydrogène (H_2O_2) par le GPO.
Au final, le peroxyde d'hydrogène (H_2O_2) réagit avec du 4-aminophénazone (4-AF) et du p-chlorophénol, réaction catalysée par la peroxydase (POD), ce qui donne une couleur rouge:

$$\text{Triglycérides} + H_2O \xrightarrow{\text{LPL}} \text{Glycérol} + \text{Acides gras libres}$$

$$\text{Glycérol} + \text{ATP} \xrightarrow{\textit{Glycérolkinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AF} + \text{p-Chlorophénol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

L'intensité de la couleur formée est proportionnelle à la concentration de triglycérides présents dans l'échantillon testé [1, 2,3].

SIGNIFICATION CLINIQUE

Les triglycérides sont des graisses qui fournissent à la cellule son énergie. Tout comme le cholestérol, ils sont transportés vers les cellules de l'organisme par les lipoprotéines du sang.
Un régime fort en graisses saturés ou en carbohydrates peut élever les niveaux de triglycérides.
Leur augmentation est relativement neutre. Diverses maladies, telles que certaines dysfonctions hépatiques (cirrhose, hépatite, obstruction biliaire) ou diabètes mellitus, peuvent être associées à des hausses de triglycérides[3, 6, 7,].
Le diagnostique clinique doit tenir compte des données cliniques et de laboratoire.

REACTIFS

R 1 Tampon	GOOD pH 7,5 p-Chlorophénol	50 mmol/L 2 mmol/L
R 2 Enzymes	Lipoprotéine lipase (LPL) Glycérol kinase (GK) Glycérol-3-oxydase (GPO) Peroxydase(POD) 4 – Aminophénazone (4-AF) ATP	150000 U/L 500 U/L 2500 U/L 440 U/L 0,1 mmol/L 0,1 mmol/L
TRIGLYCERIDES CAL	Patron primaire de détection de triglycérides 200 mg/dL	

PREPARATION

Réactif de travail (RT): Dissoudre (→) le contenu d'une capsule d'enzymes R 2 et un flacon de tampon R 1.
Réf: 1001310 Réactif de travail (RT): Reconstituer (→) le contenu d'une capsule d'enzymes R 2 dans 10 mL de tampon R 1.
Refermer et agiter doucement jusqu'à ce que le contenu soit dissout. Stabilité du R:: 6 semaine au réfrigérateur (2-8ºC) ou une semaine à 15-25ºC.

CONSERVATION ET STABILITE

Tous les composants du kit sont stables jusqu'à la date de péremption indiquée sur l'étiquette, et si les flacons sont maintenus hermétiquement fermés à 2-8ºC, à l'abri de la lumière et des sources de contamination. Ne pas utiliser les réactifs en dehors de la date indiquée.
Indices de détérioration des réactifs:
- Présence de particules et turbidité.
- Absorbation (A) du blanc à 505 nm ≥ 0,14.

MATERIEL SUPPLEMENTAIRE

- Spectrophotomètre ou analyseur pour les lectures à 505 nm.
- Cuvettes de 1,0 cm d'éclairage.
- Equipement classique de laboratoire.

ECHANTILLONS

Sérum ou plasma héparinisé ou EDTA [1]. Stabilité de l'échantillon : 5 jours à 2-8ºC.

PROCEDURE

1. Conditions de test:
   Longueur d'ondes: . . . . . 505 nm (490-550)
   Cuvette:. . . . . 1 cm d'éclairage
   Température 37ºC/.15-25ºC
2. Régler le spectrophotomètre sur zéro en fonction de l'eau distillée
3. Pipetter dans une cuvette:

	Blanc	Modèle	Echantillon
RT (mL)	1,0	1,0	1,0
Modèle [(Remarque 1, 2)] (µL)	--	10	--
Echantillon (µL)	--	--	10

4. Mélanger et incuber 5 minutes a 37ºC ou 10 min. à température ambiante.
5. Lire l'absorbation (A) du patron et l'échantillon, en comparaison avec le blanc du réactif. La couleur reste stable pendant au moins 30 minutes.

CALCULS

$$\frac{(A)\text{Echantillon}}{(A)\text{ Modèle}} \times 200 \text{ (modèle conc.)} = \text{mg/dL de triglycéride dans l'échantillon}$$

Facteur de conversion: mg/dL x 0,0113 = mmol/L.

CONTROLE DE QUALITE

Il est conseillé d'analyser conjointement les échantillons de sérum dont les valeurs ont été contrôlées: SPINTROL H Normal et pathologique (Réf. 1002120 et 1002210).
Si les valeurs se trouvent en dehors des valeurs tolérées, analyser l'instrument, les réactifs et le calibreur.
Chaque laboratoire doit disposer de son propre contrôle de qualité et déterminer les mesures correctives à mettre en place dans le cas où les vérifications ne correspondraient pas aux attentes.

VALEURS DE REFERENCE

Hommes: 40 – 160 mg/dL
Femmes: 35 – 135 mg/dL
Ces valeurs sont données à titre d'information. Il est conseillé à chaque laboratoire de définir ses propres valeurs de référence.

CARACTERISTIQUES DE LA METHODE

Gamme de mesures: Depuis la limite de détection *de* 0,000 mg/dL jusqu'à la limite de linéarité de 2200 mg/dL.
Si la concentration de l'échantillon est supérieure à la limite de linéarité, diluer 1/2 avec du ClNa 9 g/L et multiplier le résultat final par 2.
Précision:

	Intra-série (n=20)		Inter-série (n=20)	
Moyenne (mg/dL)	103	219	103	217
SD	0,41	0,93	3,74	7,80
CV (%)	0,39	0,43	3,62	3,59

Sensibilité analytique: 1 mg/dL = 0,00137 A.
Exactitude: Les réactifs SPINREACT (y) ne montrent pas de différences systématiques significatives lorsqu'on les compare à d'autres réactifs commerciaux (x).
Les résultats obtenus avec 50 échantillons ont été les suivants:
Coefficient de corrélation (r): 0,99760.
Equation de la Coubre de régression: y=0,905x +10,77.
Les caractéristiques de la méthode peuvent varier suivant l'analyseur employé.

INTERFERENCES

Aucune interférence n'a été relevée avec bilirubine jusqu'à 170 µmol/L et hémoglobine jusqu'à 10 g/L.[2].
Différentes drogues ont été décrites ainsi que d'autres substances qui peuvent interférer lors de la détermination de la triglycérides [4,5].

REMARQUES

1. TRIGLYCERIDES CAL: Etant donné la nature du produit, il est conseillé de manipuler le produit avec une grande précaution. En effet, il peut être contaminé très facilement.
2. Du LCF(*Lipid Clearing Factor*) est intégré au réactif.
3. Le calibrage au moyen du patron de détection peut donner lieu à des erreurs systématiques lors de méthodes automatiques. Dans de tels cas, il est conseillé d'utiliser des calibrages sériques
4. Utiliser des embouts de pipettes jetables propres pour diffuser le produit.
5. **SPINREACT dispose de consignes détaillées pour l'application de ce réactif dans différents analyseurs.**

BIBLIOGRAPHIE

1. Buccolo G et al. Quantitative determination of serum triglycerides by use of enzimes. Clin Chem 1973; 19 (5): 476-482.
2. Fossati P et al. Clin. Chem 1982; 28(10): 2077-2080.
3. Kaplan A et al. Tryglycerides. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 437 and Lipids 1194-1206.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

PRESENTATION

Ref: 1001310		R1: 1 x 50 mL, R2: 5 → 10 mL, CAL: 1 x 5 mL
Ref: 1001311		R1: 10 x 20 mL, R2: 10 → 20 mL, CAL: 1 x 5 mL
Ref: 1001312	Cont.	R1: 10 x 50 mL, R2: 10 → 50 mL, CAL: 1 x 5 mL
Ref: 1001313		R1: 4 x 125 mL, R2: 4 → 125 mL, CAL: 1 x 5 mL
Ref: 1001314		R1: 4 x 250 mL, R2: 4 → 250 mL, CAL: 1 x 5 mL

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Liquid Reagent Ready to use for:
SpinTech240
BIOLIS 24i Premium
Prestige 24i
SAPPHIRE

CE

APO A-I

Apolipoprotein A-I

Turbidimetry

Quantitative determination of apolipoprotein A-I (APO A-I) IVD

Store 2 - 8ºC.

PRINCIPLE OF THE METHOD

Turbidimetric test for the measurement of apolipoprotein A-I in human serum or plasma.
Anti- Apo A-I antibodies when mixed with samples containing Apo A-I , form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo A-I concentration of the patient sample, that can be quantified by comparison from a calibrator of known Apo A-I concentration.

CLINICAL SIGNIFICANCE[1]

Apo A-I is the major structural apolipoprotein in HDL and constitutes about 70% of the total protein. Apo A-I is a cofactor for lecithin-cholesterol-acyl-transferase (LCAT), the enzyme responsible for forming cholesteryl esters in plasma and plays and important role in the transport of cholesterol from peripheral tissues to the liver, to be finally excreted. Measurements of Apo A-I concentration is specially important in detecting coronary heart disease risk (CHD) as well as in the diagnostic of hyperlipoproteinemia. Concentrations < 120 mg/L are associated to an increased CHD risk, while concentrations ≥ 160 mg/L may even protect from the same risk. Patients with deficiencies in Apo A-I synthesis may highly increase the CHD risk.
Tanger disease, a consequence of an Apo A-I catabolism defect, is characterized by several reduced plasma HDL cholesterol (HDL-c) concentration, abnormal HDL composition and accumulation of cholesteryl esters in many body tissues. Plasma HDL-c and Apo A-I concentrations in homozygotes are very low, while Apo A-II concentration is less than 10% of its normal concentration. Heterozygotes are characterized by half-normal concentration of HDL-c, Apo AI and Apo –II. Current evidence suggests that these patients have increased incidence of CHD.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/L, PEG , pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human Apo B, tris 50 mmol/L, pH 7.5. Sodium azide 0.95 g/L.
Optional	APO CAL ref: 93005

CALIBRATION

The assay and the value of the calibrator concentration have been standardized against the Certified Reference Material WHO/IFCC SP1-01 (CDC, USA). It is recommended the use of the APO CAL Calibrator for calibration.

PREPARATION

Reagents: Ready to use.
Calibration Curve: Prepare the following APO CAL Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the Apo A-I calibrator by the corresponding factor stated in table bellow to obtain the Apo A-I concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.
Reagent deterioration: The presence of particles and turbidity.
Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Spintech 240 autoanalizer
- Laboratory equipment.

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 2 weeks at 2-8ºC or 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

SPINTECH 240 APPLICATION

Item Name APO A1

DATA INFORMATION
Units mg/dL
Decimals 0

ANALYSIS
Type END
W.Length 1 340
Method Turbidimetry
SLOPE INTER
1.000 x + 0

CALIBRATION
TYPE Spline

STANDARD
#1 0.10 x Cal. Val #4 0.75 x Cal. Val
#2 0.25x Cal. Val #5 1.00 x Cal. Val
#3 0.50 x Cal. Val #6

NORMAL RANGE (37ºC)
LOW HIGH
SERUM MALE
FEMALE
URINE

Item Name APO A1

ASPIRATION
KIND Single ✔ Double

VOLUME**
SAMPLE 2 µL
REAGENT 1 240 µL
REAGENT 2 60 µL

Third Mix ✔ OFF ON
R1 Blank ✔ Water R1-B

MONITOR
0 LEVEL POINT 1
SPAN 3.000

DATA PROCESS
READ
START END
MAIN 42 43
SUB 30 31

ABSORBANCE LIMIT
LOW -3.000
HIGH 3.000

ENDPOINT LIMIT 3
LINEAR CHECK (%)

FACTOR
Blank Correction 1.000

PROZONE CHECK
START END LIMIT (%)
FIRST
SECOND ✔ Low High
THIRD ✔ Low High

*** Modify reagents and sample volumes according to the range accepted but keeping always the mentioned ratio.*

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Spinreact Apolipoprotein Control (Ref.:93006) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]

Between 122 – 161 mg/dL.

Each laboratory should establish its own reference range.

INTERFERENCES

Hemoglobin (20 g/L), bilirrubin (40 mg/dL), lipemia (< 5 g/L), and rheumatoid factor (800 IU/mL) do not interfere. Other substances may interfere [6,7].

NOTES

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
3. Rifai N Arch Pathol Lab Med 1986: 110: 694-701.
4. Freedman DS et al. N Eng J Med 1986; 315: 721-726.
5. Sakurabayashi I et al. Clinica Chimica Acta 2001; 312: 87-95.
6. Young DS.Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

PACKAGING

Ref.: TK1103012	Cont.	R1. Diluent: 2 x 24 mL R2. Antibody: 2 x 6 mL

SPINREACT,S.A./S.A.U. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
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APO-B

Apolipoprotein B

Turbidimetry

Quantitative determination of apolipoprotein B (APO B)
IVD

Store 2 - 8ºC.

PRINCIPLE OF THE METHOD

Turbidimetric test for the measurement of apolipoprotein B in human serum or plasma.
Anti- Apo B antibodies when mixed with samples containing Apo B, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Apo B concentration of the patient sample, that can be quantified by comparison from a calibrator of known Apo B concentration.

CLINICAL SIGNIFICANCE[1]

Apo B is the major structural apolipoprotein in VLDL (Very Low Density Lipids), LDL (Low Density Lipids) lipoproteins and chylomicron. The most important function is the transport of rich tryglicerides lipoproteins from liver and intestine to other tissues. Apo B exists in two forms: Apo B-100 and Apo B-48. Apo B-100, the most important component of LDL, is synthesized in the liver and excreted in plasma as part of VLDL. Apo B-48, the most important component of chylomicrons, is synthesized in the intestine.
Several studies demonstrated that in people with coronary heart disease (CHD), changes in the serum concentrations of Apo A-I and Apo B are similar to those for HDL and LDL, respectively and whereas, are somewhat better discriminators of people with CHD than the LDL and HDL cholesterol concentrations.
The hiperbetalipoproteinemia is characterized by increased LDL Apo B-100 concentrations with normal or moderately increased concentrations of LDL cholesterol. The ratio of LDL cholesterol to Apo B-100 is therefore reduced in these patients.
Defects in the Apo B structure or lipoproteins containing Apo B prevent the secretion of triglycerides rich intestinal and hepatic lipoproteins; this disorder occurs in abetaliporpteinemia or homozygous hypobetalipoproteinemia.

REAGENTS

Diluent (R1)	Tris buffer 20 mmol/L, PEG , pH 8.3. Sodium azide 0.95 g/L.
Antibody (R2)	Goat serum, anti-human Apo B, tris 50 mmol/L, pH 7.5. Sodium azide 0.95 g/L.
Optional	APO CAL ref: 93005

CALIBRATION

The assay and the value of the calibrator concentration have been standardized against the Certified Reference Material WHO/IFCC SP3-07 (CDC, USA). It is recommended the use of the APO CAL Calibrator for calibration. The reagent (both monoreagent and bireagent) should be recalibrated every three weeks, when the controls are out of specifications, and when changing the reagent lot or the instrument settings. For monoreagent, a reagent blank should be run daily before sample analysis.

PREPARATION

Reagents: Ready to use.
Calibration Curve: Prepare the following APO CAL Calibrator dilutions in NaCl 9 g/L as diluent. Multiply the concentration of the Apo B calibrator by the corresponding factor stated in table bellow to obtain the Apo B concentration of each dilution.

Calibrator dilution	1	2	3	4	5	6
Calibrator (µL)	--	10	25	50	75	100
NaCl 9 g/L (µL)	100	90	75	50	25	-
Factor	0	0.1	0.25	0.5	0.75	1.0

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use reagents over the expiration date.
Reagent deterioration: The presence of particles and turbidity.
Do not freeze; frozen Antibody or Diluent could change the functionality of the test.

ADDITIONAL EQUIPMENT

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 340 nm filter.

SAMPLES

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 2 weeks at 2-8ºC or 3 months at –20ºC.

The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

PROCEDURE

1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength : 340 nm
   Temperature : 37 ºC
   Cuvette ligth path : 1cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Reagent R1 (µL)	800
Sample or Calibrator (µL)	7

5. Mix and read the absorbance (A_1) after the sample addition.
6. Immediately, pipette into de cuvette:

Reagent R2 (µL)	200

7. Mix and read the absorbance (A_2) of calibrators and sample exactly 2 minutes after the R2 addition.

Spinreact has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCULATIONS

Calculate the absorbance difference (A_2-A_1) of each point of the calibration curve and plot the values obtained against the Apo B concentration of each calibrator dilution. Apo B concentration in the sample is calculated by interpolation of its (A_2-A_1) in the calibration curve.

QUALITY CONTROL

Control sera are recommended to monitor the performance of manual and automated assay procedures. Spinreact Apolipoprotein Control (Ref.:93006) is available. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]

Between 69 – 105 mg/dL.
Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1. **Measurement range:** Up to 250 mg/dL, under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. **Detection Limit:** Values less than 3.02 mg/dL give non-reproducible results.
3. **Precision:** The reagent has been tested for 20 days, using three levels of serum in a EP5-based study (NCCLS).

EP5	CV (%)		
	23.92 mg/dL	59.08 mg/dL	119.07 mg/dL
Total	7.4%	4.3%	3.6%
Within Run	2.0%	1.4%	1.0%
Between Run	3.7%	2.2%	1.8%
Between Day	6.1%	3.4%	3.0%

4. **Accuracy:** Results obtained using this reagent (y) were compared to those obtained with a Daiichi immunoturbidimetric method. 48 samples ranging from 50 to 200 mg/dL of Apo B were assayed. The correlation coefficient (r) was 0.982 and the regression equation y = 0.996x + 5.112.

The results of the performance characteristics depend on the used analyzer.

INTERFERENCES

Hemoglobin (20 g/L), bilirrubin (40 mg/dL), lipemia (2.5 g/L), and rheumatoid factor (800 UI/mL) do not interfere. Other substances may interfere [6,7].

NOTES

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

BIBLIOGRAPHY

1. Clinical Guide to Laboratory Tests, Edited by NW Tietz W B Saunders Co., Phipladelphia, 483, 1983.
2. Mahley RW et al. J Lipids Res 1984; 25: 1277-1294.
3. Brown MS at al. Science 1986; 232:34-47.
4. Freedman DS et al. N Eng J Med 1986; 315: 721-726.
5. Sakurabayashi I et al. Clinica Chimica Acta 2001; 312: 87-95.
6. Young DS.Effects of disease on clinical laboratory tests, 3th ed. AACC Pres, 1997.
7. Friedman and Young. Effects of disease on clinical laboratory tests, 3tn ed. AACC Pres, 1997.

PACKAGING

Ref.: 1003013	Cont.	R1. Diluent: 1 x 40 mL R2. Antibody: 1 x 10 mL

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Annex 2

Diabète, Obésité & Métabolisme Postprandial

Formulaire

Partie réservée à l'organisateur

L'organisateur :..... N° de code ☐ N° de formulaire ☐

L'organisateur :.....

Date de remise : Jour ☐☐ Mois ☐☐ Année ☐☐☐☐

Date de récupération : Jour ☐☐ Mois ☐☐ Année ☐☐☐☐

Identification du répondant

بطاقة تعريفية

Nom :..... **اللقب:**.....

Prénom : ..... **الاسم:**.....

Sexe : M☐ F☐ **الجنس:** ذكر☐ أنثى☐

Date de naissance : ☐☐ ☐☐ ☐☐☐☐ **تاريخ الميلاد :** ☐☐ ☐☐ ☐☐☐☐

Adresse personnelle : ..... **العنوان الشخصي:**.....

....

N° Téléphone : ☐☐☐☐☐☐☐☐☐☐ **رقم الهاتف:** ☐☐☐☐☐☐☐☐☐☐

E-mail (@) :..... **البريد الالكتروني (@):**.....

DONNEES SOCIOPROFESSIONNELLES — المعلومات الاجتماعية المهنية

- **Statut civil :**
  -Marié ☐ -Divorcé ☐
  -Veuf (ve) ☐ -Célibataire ☐

- **الحالة العائلية:**
  - متزوج ☐ - مطلق ☐
  - أرمل ☐ - أعزب ☐

- Nombre d'enfants :……..

- عدد الأبناء:.....

- **Niveau d'étude :**
  -Sans ☐ -Primaire ☐
  -Moyen ☐ -Secondaire ☐
  -Supérieur ☐

- **المستوى الدراسي:**
  - بدون مستوى ☐ - الابتدائي ☐
  - المتوسط ☐ - الثانوي ☐
  - العالي ☐

- **Profession :**
  -Occupez-vous un emploi ?
  -Oui ☐ -Non ☐
  -Horaires de travail :
  -Nocturne ☐ -Diurne ☐

- **الوظيفة:**
  -هل تشغل أي وظيفة؟
  -نعم ☐ -لا ☐
  - ساعات العمل:
  - في الليل ☐ - في النهار ☐

- **Activité professionnelle :**
  - Employé (e) de l'Etat ☐
  - Employé dans le privé ☐
  - Indépendant (e) ☐
  - Bénévole ☐
  - Etudiant (e) ☐
  - Maître (sse) de maison ☐
  - Retraité (e) ☐
  - Chômeur (se) ☐
  - Autres ……
- **Logement :**
  - Immeuble ☐
  - Villa ou étage de villa ☐
  - Maison traditionnelle ☐
  - Habitat précaire ☐
  - Autres ……
- **Résidence :**
  - Urbaine ☐ -Rurale ☐

- **المهنة:**
  - موظف (ة) في الدولة ☐
  - موظف في القطاع الخاص ☐
  - خاص ☐
  - متطوع ☐
  - طالب (ة) ☐
  - رب (ة) منزل ☐
  - متقاعد (ة) ☐
  - عاطل (ة) عن العمل ☐
  - أخرى.....
- **المسكن:**
  - عمارة ☐
  - فيلا أو طابق فيلا ☐
  - بيت تقليدي ☐
  - بيت غير مستقر ☐
  - أخرى.....
- **الإقامة:**
  -منطقة حضرية ☐ -منطقة ريفية ☐

MODE DE VIE — نمط الحياة

- Est-ce que vous êtes fumeur ?
  Oui ☐ Non ☐ Ex-fumeur ☐
- Si oui, depuis quand ? ⌊_⌊_⌋ ans
- Quelle est la quantité moyenne par jour ? ⌊_⌊_⌋
- Prenez vous des boissons alcoolisées ?
  Oui ☐ Non ☐
- Si oui, à quelle fréquence ? ⌊_⌊_⌋
- Avez-vous une allergie quelconque ?

……

- Faites vous une activité physique régulière ?
  Oui ☐ Non ☐ Laquelle ?.....
- Si oui, combien de jours par semaine ? ⌊_⌊_⌋j
- Combien de temps y consacrez-vous ? ⌊_⌊_⌋h
- Combien de fois vous vous pesez ? ⌊_⌊_⌋ /mois

- هل تدخن؟
  نعم ☐ لا ☐ مدخن سابق ☐
- إذا كانت الإجابة بنعم، منذ متى؟ ⌊_⌊_⌋ سنوات
- ما هو متوسط عدد السجائر في اليوم الواحد؟ ⌊_⌊_⌋
- هل تشرب المشروبات الكحولية؟
  نعم ☐ لا ☐
- إذا كانت الإجابة بنعم، ما هو المعدل المعتاد؟ ⌊_⌊_⌋
- هل لديك أي نوع من الحساسية؟

.....

- هل تمارس الرياضة بانتظام؟
  نعم ☐ لا ☐ ما نوعها؟ .....
- إذا كانت الإجابة بنعم، كم عدد أيام في الأسبوع؟ ⌊_⌊_⌋يوم
- كم من الوقت تخصص لها؟ ⌊_⌊_⌋ ساعة
- كم من مرة تزن جسمك؟ ⌊_⌊_⌋ في الشهر

- **Historique du patient :**

- Depuis quand êtes-vous diabétique? ⌊_⌊_⌋ ans
- Comment vous en êtes vous rendu compte?

……

- Cela a-t-il changé votre vie ?

Sport ☐ Sorties ☐ Etudes ☐ travail ☐

- Depuis quand êtes-vous en surpoids ? ⌊_⌊_⌋ ans
- Modification du poids (6 dernier mois)
  Perte ☐ Gain ☐ Pas de changement ☐
- Suivez-vous votre glycémie régulièrement ?
  Oui ☐ Non ☐
- Si oui, à quelle fréquence ? ⌊_⌊_⌋ / jour
- Avez-vous un parent (1er ou 2ème degré) ou un frère (sœur) vivant ou décédé souffrant ou ayant souffert de :
  Hypertension ☐ Surpoids (obésité) ☐
  Diabète ☐ Maladie cardiaque ☐

-منذ متى أنت مصاب بالسكري؟ ⌊_⌊_⌋ سنوات
-كيف اكتشفت انك مصاب بالسكري؟

.....

-هل غير مرض السكري من مجرى حياتك؟
الرياضة ☐ الخروج ☐ الدراسة ☐ العمل ☐

-منذ متى تعاني من زيادة الوزن؟ ⌊_⌊_⌋ سنوات
-هل تغير وزنك في الستة اشهر الاخيرة؟
زيادة ☐ خسارة ☐ لا تغيير ☐
-هل تتابع نسبة السكر في الدم بشكل منتظم؟
نعم ☐ لا ☐
-إذا كانت الإجابة بنعم، كم مرة؟ ⌊_⌊_⌋ مرة في اليوم
-هل لديك والد (ة) (الدرجة الأولى أو الثانية) أو أخ (أخت) أحياء أو متوفين يعاني أو عانى من:
ارتفاع ضغط الدم ☐ مرض السكري ☐
زيادة الوزن (السمنة) ☐ مرض القلب ☐

COMPORTEMENT ALIMENTAIRE ET HYGIENE

- Combien de repas prenez-vous par jour ? |__|__|
- A quelle heure prenez vous ?
  le petit déjeuner |__|__|__|__| le diner |__|__|__|__|
- Est-ce que vous mangez à des heures fixes ?
  Oui ☐ Non ☐
- Vous arrive-t-il souvent de supprimer un repas ?
  Oui ☐ Non ☐ lequel ?.....
- Vous arrive-t-il de vous levez la nuit pour manger ?
  Oui ☐ Non ☐
- Prenez vous certaines collations pendant la journée ?
  Oui ☐ Non ☐
- Évitez-vous un certain type d'aliment ?
  Oui ☐ Non ☐ pourquoi ?.....
- Vous mangez seul ou avec autrui ?.....
- Est-ce que vous préférez manger ;
  à la maison ☐ au restaurant ☐
- Quelle est la durée moyenne du :
  Petit déjeuner |__|__|mn
  Déjeuner |__|__|mn
  Gouter |__|__|mn
  Dîner |__|__|mn

- A quelle heure vous levez vous le matin ? |__|__|
- Souffrez-vous de troubles digestifs ?
  Oui ☐ Non ☐
- Avez-vous faim pendant la journée ?
  Oui ☐ Non ☐ combien de fois ? |__|__|
- Mangez-vous dès que vous avez faim ?
  Oui ☐ Non ☐
- Limitez vous volontairement votre alimentation pendant les repas ?
  Oui ☐ Non ☐
- Pouvez-vous ne pas manger pendant 4 heures ?
  Oui ☐ Non ☐
- Que buvez-vous habituellement au cours des repas ?
  Eau du robinet ☐
  Eau minérale ☐
  Soda ☐
  Boissons fruitées ☐
  autres ☐

السلوك الغذائي و الوقاية

-كم عدد الوجبات التي تتناولها يوميا؟|__|__|
-في أي وقت تتناول وجبة؟
الإفطار|__|__| العشاء |__|__|
-هل تناول الوجبات في أوقات محددة؟
نعم ☐ لا ☐
-هل يحدث و أن تتخلى عن وجبة ؟
نعم ☐ لا ☐ أي وجبة؟.....
-هل تقوم أثناء الليل لتناول الطعام؟
نعم ☐ لا ☐
-هل تتناول بعض الوجبات الخفيفة خلال اليوم؟
نعم ☐ لا ☐
-هل تتجنب نوع معين من الطعام؟
نعم ☐ لا ☐ لماذا؟.....
-أنت تأكل وحدك أو مع الآخرين؟..... .....
-هل تفضل أن تأكل؛
في المنزل ☐ في مطعم ☐
-ما هو متوسط مدة:
الإفطار |__|__| دقيقة
الغداء |__|__| دقيقة
وجبة خفيفة |__|__| دقيقة
العشاء |__|__| دقيقة

-على أي ساعة تستيقظ صباحا؟ |__|__|
-هل تعاني من اضطرابات في الجهاز الهضمي؟
نعم ☐ لا ☐
-هل تشعر بالجوع خلال النهار؟
نعم ☐ لا ☐ كم مرة؟ |__|__|
-هل تأكل عندما تحس بالجوع؟
نعم لا
- هل تحدد طواعية من تناول الأطعمة أثناء الوجبات الغذائية؟
نعم ☐ لا ☐
-هل تستطيع عدم تناول الطعام لمدة 4 ساعات؟
نعم ☐ لا ☐
-ماذا تشرب عادة خلال الوجبات؟
ماء الحنفية ☐
مياه معدنية ☐
مشروبات غازية ☐
مشروبات فواكه ☐
اخرى ☐

QUESTIONS SUR LE JEUNE DE RAMADAN

- Pratiquez-vous le jeûne du mois de Ramadan de façon régulière ?
  Oui ☐ Non ☐
- Qui c'est la personne qui vous a recommandé de jeûner ?
  Votre médecin ☐
  Un diabétique ☐
  Autres personne ☐
- Vous arrive-t-il d'abandonner le jeûne au cours du mois ?
  Oui ☐ Non ☐
- Faites-vous des contrôles réguliers de poids et de la glycémie durant ce mois ?
  Oui ☐ Non ☐

أسئلة خاصة بصيام شهر رمضان

- هل تصوم رمضان بصفة منتظمة ؟
نعم ☐ لا ☐
- من أوصاك بصيام شهر رمضان؟ -
الطبيب ☐
مريض بالسكري ☐
شخص اخر ☐
- هل حدث لك و ان قطعت الصيام خلال شهر رمضان؟
نعم ☐ لا ☐
هل تراقب نسبة السكر في الدم و وزنك بصفة منتظمة خلال شهر رمضان؟
نعم ☐ لا ☐

Merci pour votre collaboration

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Annex 3

ljm Libyan Journal of Medicine

co-action PUBLISHING

ORIGINAL ARTICLE

Impact of corpulence parameters and haemoglobin A1c on metabolic control in type 2 diabetic patients: comparison of apolipoprotein B/A-I ratio with fasting and postprandial conventional lipid ratios

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Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University, Sidi-Bel-Abbes, Algeria

Background and objective: The incidence of diabetes co-morbidities could probably be better assessed by studying its associations with major corpulence parameters and glycaemic control indicators. We assessed the utility of body mass index (BMI), waist circumference (WC), and glycosylated haemoglobin (HbA1c) levels in metabolic control for type 2 diabetic patients.

Methods: Fasting and postprandial blood samples were collected from 238 type 2 diabetic patients aged 57.4 ± 11.9 years. The sera were analysed for glucose, HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and apolipoproteins (apoA-I and apoB). Ratios of lipids and apolipoproteins were calculated and their associations with BMI, WC, and HbA1c levels were analysed.

Results: Our investigation showed increases in most fasting and postprandial lipid parameters according to BMI and WC. In men, postprandial HDL-c and TG levels were significantly higher ($p < 0.05$) in overweight and obese patients, respectively, as well as in patients with abdominal obesity. Contrariwise, postprandial TC levels were significantly higher ($p < 0.01$) in overweight and abdominal obese women. However, elevations of apoA-I and apoB levels were according to BMI and WC in both genders. There was a strong influence of BMI, WC, and HbA1c levels on the apoB/apoA-I ratio compared to traditional fasting and postprandial lipid ratios in both men and women. The apoB/apoA-I ratio was more correlated with postprandial TC/HDL and LDL-c/HDL-c ratios in men and with postprandial TG/HDL-c in women.

Conclusion: The apoB/apoA-I ratio is helpful in assessing metabolic risk caused by overall obesity, abdominal obesity and impaired glycaemia in type 2 diabetic patients.

Keywords: *type 2 diabetes*; *body mass index*; *waist circumference*; *HbA1c*; *metabolic control*

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Recent estimates state that the worldwide prevalence of type 2 diabetes is increasing and is likely to affect over 400 million people by 2030. This rising prevalence of type 2 diabetes is thoroughly linked to the upsurge in obesity. More than 90% of type 2 diabetes is attributable to excess weight (1).

Measured by body mass index (BMI), obesity is regarded as a powerful risk factor for type 2 diabetes and its co-morbidities and has increased clearly during the past decades. Estimates suggest that obesity will affect up to 1 billion people in 2030 (2). Although the BMI is one of the most commonly used indicators of overweight and obesity, it does not take into account the body fat pattern (3). Independently from overall obesity, assessment of abdominal obesity by waist circumference (WC) has been shown to be a strong risk factor for type 2 diabetes as well. These obesity indicators may vary according to some factors such as ethnicity, age, and sex (4, 5). Among Europeans and the white US population, general obesity is considered as a consistent predictor of diabetes. However, central obesity has been shown to be a better predictor in Asian populations (6, 7). In contrast, in 2004, Janssen et al. (8) showed that obesity-related health risk is explained by WC and not by BMI.

Regardless of BMI and WC as powerful indicators of obesity, assessment of glucose and lipid levels appears to identify diabetic individuals at increased risk for development of cardiovascular diseases (CVD). Glycosylated haemoglobin (HbA1c) is considered as a long-term marker for blood glucose fluctuations. Elevated HbA1c is an independent risk factor for coronary heart disease (CHD) in patients with or without diabetes (9). However, and contrary to apolipoproteins, studies suggest that lipid levels may not vary much between the fasting and the postprandial periods and that the risk of CHD and stroke is similarly increased for both postprandial and fasting lipid rates (10).

This study was conducted on patients with type 2 diabetes to evaluate, during both fasting and postprandial states, the impact of overweight/obesity (identified through BMI), abdominal fat accumulation (measured by WC), and HbA1c levels (in mmol/mol) on glucose and lipid blood parameters, and also on dyslipidaemia by studying the mutual association between traditional lipid ratios and apolipoproteins ratios.

Patients and methods

Study population

A total of 238 type 2 diabetic patients (86 males and 152 females) were enrolled in this study. Our investigation lasted 9 months, from March to December 2013, at the level of the Public Establishment of Local Health Centre (Diabetes Centre of Ex Gambetta and Mostefa Ben Brahim Polyclinic) in Sidi-Bel-Abbes city and Meslem Tayeb Hospital in Mascara city. These two cities are located in the north-western region of Algeria.

According to data available at the health departments of these two cities, the approximate number of patients with type 2 diabetes was about 22,000 (according to the census of December, 2012), of whom two-thirds were women and almost 18% were treated solely with oral anti-diabetic agents.

During periodic medical follow-up sessions and based on a careful analysis of their medical records, we randomly solicited type 2 diabetic patients who met the inclusion criteria, that is, aged between 19 and 75 years, diabetes duration of less than 15 years, not suffering from diabetes complications, and exclusively under oral anti-diabetic treatments. We used the simple random sampling method without replacement, in which each individual in the targeted population has the same probability of being selected.

The mean ages of male and female patients were 56.8 ± 13.1 and 57.7 ± 11.3 years, respectively. The average diabetes duration was 6.8 ± 3.7 years (6.3 ± 3.4 years for males and 7.1 ± 3.9 years for females). The most common anti-diabetic agents prescribed for our patients were metformin alone (38.7%) or in combination with glimepiride (55.9%), followed by sulfonylureas (5.4%). Participants were asked to abstain from their oral anti-diabetic medications during the day of blood sampling. Patients treated with insulin or using lipid-lowering drugs during the study, as well as pregnant women, were excluded from this study. For data collection, we used a structured questionnaire to get necessary information about general habits and a 'three-day food diary' to assess the dietary intake of each patient. The diaries were analysed using the software program Nutri Survey for windows 2007, SEAMEO-TROPMED RCCN-University of Indonesia (results of food diaries will be published later). Furthermore, a signed informed consent was obtained from all patients and their treating physicians before starting the study protocol, considering the ethical approval No. 142 dated 13 February 2013 from 'the Director of Health and Population of the *Wilaya* of Sidi-Bel-Abbes (Algeria) according to Article 25 of Decree No. 387 of 31 July 2006 about clinical trials'.

Anthropometric measurements

Body weight (in kilograms) was measured using an electronic balance (TS-2003A: 360 lb, Capacity: 180 kg, Graduations 0.1 kg) and height (in metres) was measured with a body meter (Seca 206, Germany; measuring range 0–220 cm, graduation length 1 mm). The BMI was calculated as weight (kg)/height^2 (m^2). Patients were instructed to be lightly dressed and to respect the appropriate position for height measurement (gathered feet, straight body, heels touching the wall, and staring out the horizon).

WC was measured respecting every single cm with a measuring tape (maximum 150 cm, graduation length 1 mm). The tape was gently tightened around the patient's abdomen roughly at the horizontal line just above the uppermost lateral border of the ilium, which corresponds with the line passing through the navel in men and a bit above in women.

Blood pressure measurement

OMRON *M3* Digital Automatic Blood Pressure Monitor (Omron Healthcare, Ltd. Kyoto, Japan) was used for calculating morning blood pressure.

Blood sampling and assay methods

For fasting glucose and lipid profiles, venous blood samples were collected from each patient 12 h after an overnight fast. We did not get information about the kind of food taken before the 12 h of fasting. However, for the postprandial state, blood samples were drawn 2 h after a breakfast meal for glucose and 3–4 h for lipid parameters. The usual breakfast meal in 94% of our patients provides an average of 692.0 ± 11.0 kcal [fat: 44.1 ± 0.4 g (57%); protein: 7.6 ± 0.1 g (6%); carbohydrates 49.4 ± 0.7 g (38%)].

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Enzymatic colorimetric methods (Spinreact Reagents, Spain) (11) were used to determine the fasting and the postprandial serum concentrations of glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and direct low-density lipoprotein cholesterol (LDL-c). TC/HDL-c, TG/HDL-c, and LDL-c/HDL-c ratios were, respectively, calculated for fasting and postprandial states. The quantitative determination of apo A-I and apo B was performed using turbidimetric tests without undergoing a prior fasting period (Spinreact Reagents, Spain) (12), then the apo B/apoA-I ratio was calculated.

The HbA1c levels were determined by an ion exchange resin separation method. Patients were categorised into three groups: HbA1c <53.01 mmol/mol; HbA1c $=$ 53.01–80.34 mmol/mol; HbA1c >80.34 mmol/mol.

Statistical analysis

All data were processed and analysed by using SPSS 20.0 (Statistical *Package for the Social Sciences*, IBM Corporation; Chicago, IL, August 2011). Results are expressed as means $\pm$standard deviations. Student's *t*-test was used for comparing means values and statistical significance was set at $p=0.05$. Relationships between apolipoprotein ratios and lipid ratios were studied using Pearson correlation tests and simple linear regression tests with a confidence interval of 95%.

Results

Table 1 displays the subjects' characteristics. No difference between the two genders was observed with respect to age, diabetes duration, WC, blood pressure, duration of overweight and/or obesity, and HbA1c levels. A significant gender effect was observed for weight, height, and BMI ($p<0.05$). Furthermore, a highly significant effect of gender was noticed for fasting glycaemia but not for postprandial glucose levels.

The prevalence of overweight, obesity, and abdominal obesity was more pronounced in females than in males, whereas the prevalence of normal weight was higher in male patients (43.02%) as compared to female patients (22.36%).

Our results about risk factors and lifestyle showed that 34.9% of men were current cigarette smokers (we considered subjects who reported smoking cigarettes regularly during the year before the survey to be current smokers). Women had slightly lower educational level compared to men (48.02% of women had no formal education). Regarding the history of family diseases, diabetes was the most prevalent risk factor for both men (29.06%) and women (31.57%).

Based on the BMI, in male patients a moderate elevation of fasting glucose was noted in the normal weight group (BMI <25 kg/m^2) followed by the obese group (BMI $\geq$ 30 kg/m^2) (Table 2). The postprandial glucose level was rather higher among the overweight group (2.59 ± 1.44 g/L) and the normal weight group (2.48 ± 1.17 g/L). However, in female patients, an elevation of fasting glucose levels was observed in overweight and obese patients (1.58 ± 0.66 vs. 1.51 ± 0.62 g/L, respectively). The topmost level of postprandial blood glucose was noted in the normal weight group. There was a highly significant difference between the fasting and the postprandial glucose levels. Between genders, a significant difference in fasting glycaemia was noted for the group of normal weight patients.

On the basis of WC, significant increase of the fasting glycaemia was noted in female patients suffering from abdominal obesity (1.52 ± 0.60 g/L) compared to those with normal WC (1.34 ± 0.39 g/L). In both genders, abdominal obesity does not seem to be a factor helping to increase the blood glucose during the postprandial state.

Comparing between the fasting and postprandial states and except for HDL-c and TG, the lipid values in male patients did not differ according to the BMI and/or WC (Table 2). Significantly lower HDL-c levels were obtained during the postprandial state for BMI $=25.0$–29.9 kg/m^2 and abdominal obesity (WC >94 cm). However, TG levels showed significant increases during the postprandial state with BMI ≥30 kg/m^2 and abdominal obesity. In contrast, highly significant increases in TC were observed during the postprandial state in female patients with BMI $=25.0$–29.9 kg/m^2 ($p<0.01$) and abdominal obesity ($p<0.001$). Similarly, in women suffering from abdominal obesity, postprandial TG levels (1.65 ± 0.80 g/L) were significantly higher than fasting levels ($1.50\pm$ 0.78 g/L). The LDL-c level was lower during the postprandial state with WC ≤80 cm.

Apolipoproteins (apo A1 and apo B) showed a considerable increase according to BMI and/or WC in both genders. However, a significant effect of gender differences was noted on apo A-I in patients with BMI $=25.0$–29.9 kg/m^2 and abdominal obesity ($p<0.05$).

Table 3 shows the effect of BMI, WC, and HbA1c level on fasting lipid ratios, postprandial lipid ratios, and apolipoproteins ratios in type 2 diabetic patients. Among male patients with BMI $=25$–29.9 kg/m^2, all lipid ratios (TC/HDL-c, TG/HDL-c and LDL-c/HDL-c) increased significantly during the postprandial state compared to the fasting state. When we compared lipid ratios between fasting and postprandial states according to WC values, postprandial TC/HDL-c and TG/HDL-c ratios increased noticeably in patients with abdominal obesity (WC >94 cm). However, elevated HbA1c levels ($\geq7\%$ or ≥53.01 mmol/mol) are more likely to cause an increase in postprandial ratios of TC/HDL-c and TG/HDL-c. In female patients, only the TG/HDL-c ratio showed a significant postprandial increase in obese women (BMI ≥30 kg/m^2). Based on WC, the postprandial TC/HDL-c and TG/HDL-c ratios were significantly higher than the fasting ratios (Table 3).

Table 1. Characteristics of the patients

Variables	All patients	Males	Females	*P* value for statistical test[a]
n	238	86	152	–
Age (years), mean ± S.D.	57.36 ± 11.93	56.84 ± 13.11	57.66 ± 11.25	0.615
Duration of diabetes (years), mean ± S.D.	6.82 ± 3.74	6.33 ± 3.39	7.10 ± 3.90	0.127
Anthropometric characteristics, mean ± S.D.				
Weight (kg)	75.66 ± 13.00	78.59 ± 14.28	74.00 ± 11.94	0.009
Height (cm)	164.69 ± 8.55	171.39 ± 7.01	160.89 ± 6.85	0.000
Waist circumference (cm)	97.40 ± 13.56	95.99 ± 12.16	98.20 ± 14.28	0.229
BMI (kg/m^2)	27.89 ± 4.58	26.73 ± 4.64	28.55 ± 4.44	0.003
Overweight, obesity duration (years)	9.66 ± 6.29	10.25 ± 6.40	9.31 ± 6.25	0.463
Blood pressure, mean ± S.D.				
Systolic pressure (mmHg)	12.89 ± 1.52	12.87 ± 1.58	12.90 ± 1.49	0.875
Diastolic pressure (mmHg)	7.59 ± 0.95	7.61 ± 0.94	7.58 ± 0.96	0.821
HbA1c (mmol/mol), mean ± S.D.	59.45 ± 10.05	58.91 ± 10.38	59.78 ± 9.83	0.280
Fasting glycaemia (g/L), mean ± S.D.	1.60 ± 0.61	1.75 ± 0.62	1.51 ± 0.59	0.004
Postprandial glycaemia (g/L), mean ± S.D.	2.31 ± 1.03	2.42 ± 1.15	2.24 ± 0.96	0.200
Prevalence of weight categories, *n* (%)				
Normal weight, BMI < 25 kg/m^2	71 (29.83)	37 (43.02)	34 (22.36)	0.053
Overweight, BMI = 25.0 − 29.9 kg/m^2	92 (38.65)	26 (30.23)	66 (43.42)	0.101
Obesity, BMI ≥ 30 kg/m^2	75 (31.51)	23 (26.74)	52 (34.21)	0.104
Abdominal obesity[b]	191 (80.25)	50 (58.13)	141 (92.76)	0.028
Educational status, *n* (%)				
Without education	91 (38.23)	18 (20.93)	73 (48.02)	0.050
Primary school	43 (18.06)	18 (20.93)	25 (16.44)	0.182
Middle school	46 (19.32)	25 (29.06)	21 (13.81)	0.118
Secondary school	42 (17.64)	17 (19.76)	25 (16.44)	0.420
Higher education	16 (6.72)	8 (9.30)	8 (5.26)	0.249
Smoking, *n* (%)				
Never	200 (84.03)	48 (55.81)	152 (100)	0.024
Former	8 (3.36)	8 (9.30)	–	–
Current	30 (12.60)	30 (34.88)	–	–
Practice of sports activity, *n* (%)				
Yes	52 (21.84)	31 (36.04)	21 (13.81)	0.361
No	186 (78.15)	55 (63.95)	131 (86.18)	0.193
Family disease history, *n* (%)				
Hypertension	17 (7.14)	5 (5.81)	12 (7.89)	0.371
Overweight, obesity	33 (13.86)	16 (18.60)	17 (1.18)	0.331
Diabetes	73 (30.67)	25 (29.06)	48 (31.57)	0.252
Cardiovascular disease	17 (7.14)	4 (4.65)	13 (8.55)	0.358
All	61 (25.63)	23 (26.74)	38 (25.00)	0.407
No	37 (15.54)	13 (15.11)	24 (15.78)	0.161
Menopausal status, *n* (%)				
Pre-menopause	–	–	59 (38.81)	
Oestrogen use (yes)	–	–	42 (27.63)	–
Oestrogen use (no)	–	–	17 (11.18)	–
Post-menopause	–	–	93 (61.18)	–

[a]Comparison between males and females; mean values were compared using Student's *t*-test. Percentages were compared with Chi-square test.

[b]According to the International Diabetes Federation (IDF), waist circumference > 80 cm for women and > 94 cm for men.

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Table 2. Influence of BMI and waist circumference on fasting blood parameters, postprandial blood parameters, and apolipoproteins

	Fasting state (g/L)					Postprandial state (g/L)					Apolipoproteins (g/L)	
Variables	F Glucose	F TC	F HDL-c	F LDL-c	F TG	PP Glucose	PP TC	PP HDL-c	PP LDL-c	PP TG	apo A-I	apo B
Males ($n = 86$)												
Based on BMI												
BMI <25 kg/m^2	1.82 ± 0.71	1.66 ± 0.35	0.35 ± 0.11	1.08 ± 0.38	1.26 ± 0.65	$2.48 \pm 1.17^{***}$	1.68 ± 0.43	0.34 ± 0.12	1.07 ± 0.36	1.49 ± 0.99	1.15 ± 0.44	0.89 ± 0.23
BMI $=25.0-29.9$ kg/m^2	1.63 ± 0.63	1.66 ± 0.33	0.39 ± 0.14	1.05 ± 0.30	1.25 ± 0.55	$2.59 \pm 1.44^{***}$	1.76 ± 0.50	$0.32 \pm 0.08^{*}$	1.16 ± 0.48	1.59 ± 1.16	1.19 ± 0.34	0.84 ± 0.32
BMI ≥ 30 kg/m^2	1.79 ± 0.45	1.77 ± 0.42	0.37 ± 0.12	1.07 ± 0.35	1.37 ± 0.64	$2.15 \pm 0.64^{*}$	1.77 ± 0.46	0.38 ± 0.16	1.07 ± 0.34	$1.61 \pm 0.79^{*}$	1.29 ± 0.52	0.91 ± 0.33
Based on WC												
WC ≤ 94 cm	1.83 ± 0.66	1.63 ± 0.34	0.34 ± 0.11	1.07 ± 0.38	1.16 ± 0.54	$2.47 \pm 1.10^{***}$	1.65 ± 0.41	0.33 ± 0.13	1.06 ± 0.34	1.22 ± 0.43	1.20 ± 0.48	0.86 ± 0.23
WC >94 cm	1.69 ± 0.60	1.73 ± 0.37	0.39 ± 0.13	1.06 ± 0.32	1.38 ± 0.65	$2.39 \pm 1.20^{***}$	1.78 ± 0.48	$0.35 \pm 0.12^{*}$	1.12 ± 0.43	$1.79 \pm 1.20^{*}$	1.20 ± 0.40	0.89 ± 0.32
Females ($n = 152$)												
Based on BMI												
BMI <25 kg/m^2	$1.38 \pm 0.36^{\#}$	1.72 ± 0.31	0.39 ± 0.13	1.16 ± 0.29	1.44 ± 0.82	$2.30 \pm 0.95^{***}$	1.78 ± 0.36	0.36 ± 0.10	1.10 ± 0.28	1.58 ± 0.89	1.26 ± 0.27	0.92 ± 0.32
BMI $=25.0-29.9$ kg/m^2	1.58 ± 0.66	1.66 ± 0.36	0.40 ± 0.11	1.04 ± 0.33	1.45 ± 0.72	$2.27 \pm 0.95^{***}$	$1.81 \pm 0.50^{**}$	$0.40 \pm 0.12^{\#}$	1.08 ± 0.40	1.64 ± 0.92	$1.38 \pm 0.40^{\#}$	1.00 ± 0.52
BMI ≥ 30 kg/m^2	1.51 ± 0.62	1.72 ± 0.36	0.40 ± 0.10	1.04 ± 0.31	1.60 ± 0.77	$2.18 \pm 0.99^{***}$	1.82 ± 0.39	0.39 ± 0.11	1.07 ± 0.30	1.79 ± 0.63	1.32 ± 0.35	1.03 ± 0.46
Based on WC												
WC ≤ 80 cm	$1.34 \pm 0.39^{\#}$	1.88 ± 0.43	0.38 ± 0.17	$1.43 \pm 0.16^{\#}$	1.45 ± 0.36	$2.34 \pm 0.33^{*}$	1.64 ± 0.43	0.28 ± 0.10	$0.96 \pm 0.28^{*}$	$1.99 \pm 1.13^{\#\#}$	1.04 ± 0.23	0.82 ± 0.21
WC >80 cm	1.52 ± 0.60	1.68 ± 0.34	0.40 ± 0.10	1.04 ± 0.31	1.50 ± 0.78	$2.24 \pm 0.95^{***}$	$1.82 \pm 0.43^{***}$	$0.40 \pm 0.11^{\#}$	1.09 ± 0.34	$1.65 \pm 0.80^{*}$	$1.35 \pm 0.36^{\#}$	1.00 ± 0.47

BMI: body mass index; WC: waist circumference; F: fasting; PP: postprandial. Asterisks: Significantly different from the identical fasting parameter at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. $^{\#}$ and $^{\#\#}$: significantly different from male patients at $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

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Table 3. Variations of apolipoproteins ratio, and fasting and postprandial lipid ratios according to BMI, waist circumference, and HbA1c level

Variables	Fasting state			Postprandial state			Apolipoproteins
	TC/HDL-c	TG/HDL-c	LDL-c/HDL-c	TC/HDL-c	TG/HDL-c	LDL-c/HDL-c	apo B/apo A-I
Males ($n=86$)							
Based on BMI							
BMI <25 kg/m^2	4.95 ± 1.31	3.95 ± 2.61	3.19 ± 1.25	5.24 ± 1.30	4.94 ± 3.63	3.32 ± 1.12	0.81 ± 0.24
BMI $=25.0$–29.9 kg/m^2	4.63 ± 1.46	3.51 ± 1.72	2.99 ± 1.30	5.75 ± 2.36*	5.13 ± 3.46*	3.86 ± 2.14*	0.75 ± 0.41
BMI ≥ 30 kg/m^2	4.96 ± 1.63	3.90 ± 1.88	3.05 ± 1.30	5.00 ± 1.63	4.85 ± 2.88	3.04 ± 1.21	0.77 ± 0.30
Based on WC							
WC ≤ 94 cm	5.05 ± 1.30	3.81 ± 2.46	3.32 ± 1.32	5.24 ± 1.36	4.17 ± 2.48	3.37 ± 1.21	0.77 ± 0.25
WC >94 cm	4.71 ± 1.52	3.81 ± 1.96	2.93 ± 1.21	5.40 ± 2.02*	5.56 ± 3.78*	3.44 ± 1.74	0.79 ± 0.35
Based on HbA1c							
HbA1c <53.01 mmol/mol	5.09 ± 1.57	3.57 ± 1.74	3.41 ± 1.41	5.18 ± 1.23	4.76 ± 3.16	3.35 ± 1.16	0.80 ± 0.19
HbA1c $=53.01$–80.34 mmol/mol	4.83 ± 1.42	4.02 ± 2.30	2.98 ± 1.24	5.43 ± 2.09*	5.28 ± 3.64*	3.42 ± 1.79	0.79 ± 0.37
HbA1c >80.34 mmol/mol	4.14 ± 0.73	3.38 ± 2.84	2.62 ± 0.55	5.29 ± 1.28*	3.86 ± 1.96	3.55 ± 1.08	0.68 ± 0.26
Females ($n=152$)							
Based on BMI							
BMI <25 kg/m^2	4.89 ± 2.00	4.22 ± 2.88	3.35 ± 1.65	5.31 ± 1.88	5.08 ± 4.12	3.29 ± 1.26	0.75 ± 0.29
BMI $=25$ to 29.9 kg/m^2	4.37 ± 1.29	3.91 ± 2.22	2.76 ± 1.05	4.82 ± 1.70$^{\#}$	4.49 ± 2.95	2.92 ± 1.36$^{\#}$	0.75 ± 0.36
BMI ≥ 30 kg/m^2	4.48 ± 1.25	4.23 ± 2.38	2.74 ± 1.07	4.91 ± 1.57	4.92 ± 2.24*	2.92 ± 1.20	0.81 ± 0.37
Based on WC							
WC ≤ 80 cm	5.76 ± 2.70	4.38 ± 1.83	4.41 ± 2.02$^{\#}$	6.57 ± 2.75$^{\#}$	8.49 ± 6.35*$^{\#\#}$	3.86 ± 1.59	0.81 ± 0.24
WC >80 cm	4.43 ± 1.29	4.06 ± 2.46	2.76 ± 1.07	4.83 ± 1.53*$^{\#}$	4.48 ± 2.43*$^{\#}$	2.94 ± 1.24$^{\#}$	0.77 ± 0.36
Based on HbA1c level							
HbA1c <53.01 mmol/mol	4.78 ± 1.68	4.52 ± 2.59	3.02 ± 1.41	5.17 ± 1.96	5.62 ± 4.07*	3.04 ± 1.34	0.81 ± 0.41
HbA1c $=53.01$–80.34 mmol/mol	4.41 ± 1.26	3.93 ± 2.21	2.80 ± 1.06	4.82 ± 1.55*	4.39 ± 2.31	2.94 ± 1.27	0.74 ± 0.32
HbA1c >80.34 mmol/mol	4.41 ± 1.88	3.68 ± 3.02	2.96 ± 1.62	5.16 ± 1.65	4.38 ± 2.74	3.28 ± 1.27	0.81 ± 0.30

BMI: body mass index; WC: waist circumference. *Significantly different from the identical fasting lipid ratio at $p<0.05$. $^{\#}$ and $^{\#\#}$: significantly different from male patients at $^{\#}p<0.05$, $^{\#\#}p<0.01$.

The TC/HDL-c ratio was more influenced by a high level of HbA1c.

Between genders, appreciable lowering of TC/HDL-c, TG/HDL-c, and LDL-c/HDL-c ratios was observed in female patients suffering from abdominal obesity; low TC/HDL-c and LDL-c/HDL-c ratios were also observed in overweight women (BMI $=25.0$–29.9 kg/m^2). The apo B/apo A-I ratio did not show any significant difference between the two genders (Table 3).

As illustrated in Fig. 1, the apo B/apo A-I ratio was significantly correlated with TC/HDL-c, TG/HDL-c, and LDL-c/HDL-c ratios both in fasting and in postprandial states. However, we noticed that the apo B/apo A-I ratio provides the strongest positive correlations with TC/HDL-c ($p<0.01$, $r^2=0.321$, $F=111.453$) and LDL-c/HDL-c ($p<0.001$, $r^2=0.300$, $F=103.317$) ratios during the postprandial period.

As depicted in Table 4, we evaluated the risk of myocardial infarction in our diabetic patients in terms of increased apo B/apo A-I ratio. Tentative risk zones used in this table are based on cut-off values adapted from the Apolipoprotein-related Mortality Risk (AMORIS) (13) and INTERHEART (14) studies. For men, the proportion of patients who were likely to be exposed to a moderate or high risk was 32.6 and 26.7%, respectively. Contrariwise, for female patients, 37.5% were exposed to a higher risk of myocardial infarction.

Discussion

Diabetes mellitus is one of the most prevalent chronic diseases in almost all countries. Between 2010 and 2030, the number of adults with diagnosed diabetes is expected to increase by 69% in developing countries and by 20% in developed countries (1). Concurrently, the prevalence of obesity is increasing dramatically all over the world (15). Obesity is medically defined as a state of increased adipose tissue of a magnitude sufficient to lead to worsening of all the elements of the metabolic syndrome, namely insulin resistance, hyperinsulinaemia, dyslipidaemia, and hypertension (2, 16). The classification system for obesity is

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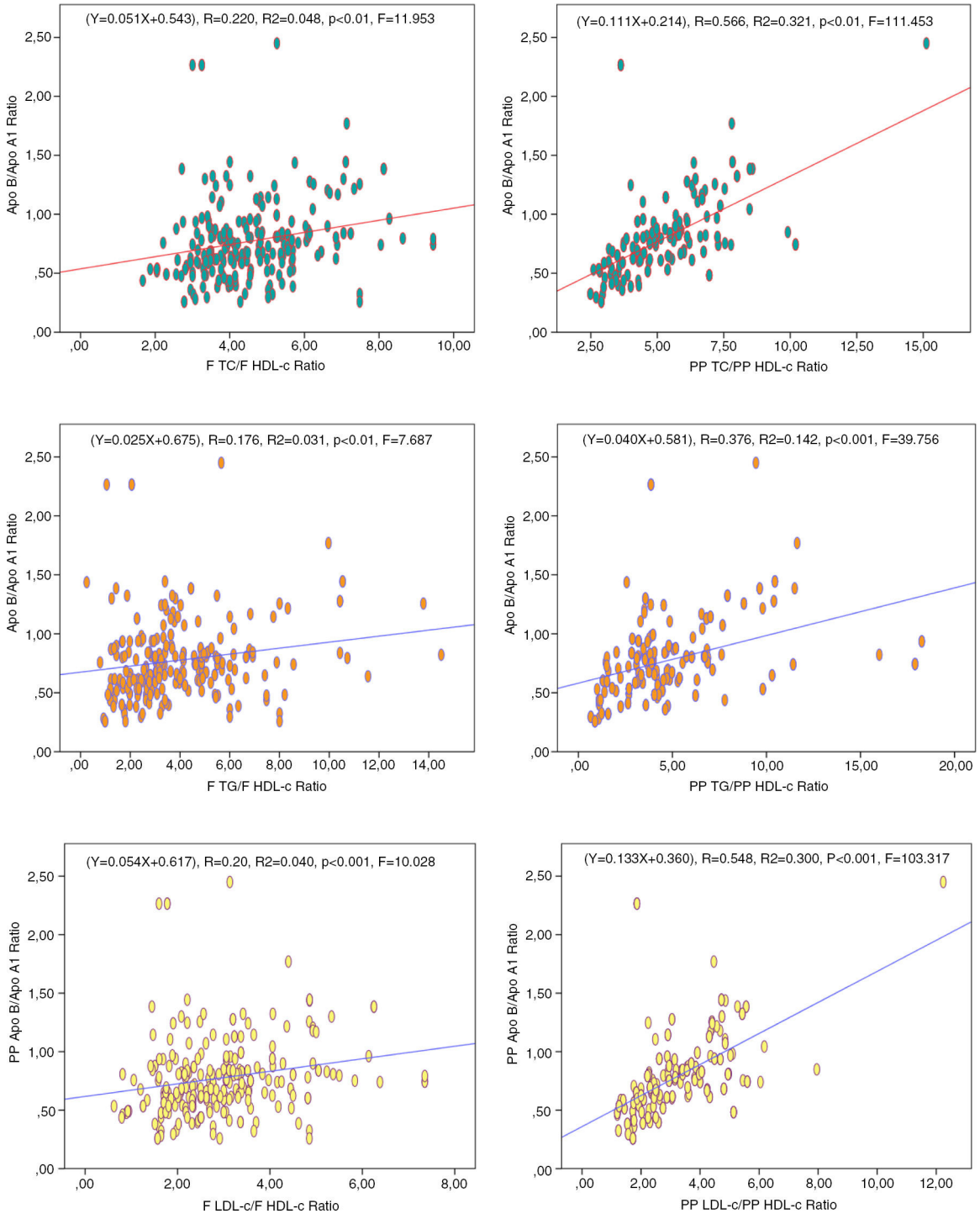

Fig. 1. Mutual association between apolipoproteins ratio and lipid ratios during fasting and postprandial states.

based on BMI as the most frequently used diagnostic tool. However, the body fat distribution, particularly abdominal fat accumulation, exerts a strong influence on the development of glucose intolerance and type 2 diabetes co-morbidities. Several investigators have reported that individuals with similar BMI but with different WC show different metabolic risks of diabetes and CVD (17, 18).

In the same context, as reported by Elley et al., HbA1c is considered as an important indicator of blood glucose control; an HbA1c 'threshold' of 53.01 mmol/mol is a significantly higher risk of macrovascular disease in diabetic patients. Every 1% higher HbA1c level above that threshold is associated with an independent increased CVD risk (19).

Table 4. Risk of myocardial infarction in terms of increased apo B/apo A-I ratio in the studied cases

	Low risk	Moderate risk	High risk
Men apo B/apo A-I ratio	0.40 – 0.69	0.70 – 0.89	0.90 – 1.10
Prevalence, *n* (%)	35 (40.69)	28 (32.55)	23 (26.74)
Women apo B/apo A-I ratio	0.30 – 0.59	0.60 – 0.79	0.80 – 1.00
Prevalence, *n* (%)	45 (29.60)	50 (32.89)	57 (37.50)

Adapted with thresholds of AMORIS and INTERHEART studies.

The purpose of the present study was to establish, during fasting and postprandial states in type 2 diabetes patients, the influence of overweight/obesity (defined by BMI), abdominal obesity (measured by WC), and HbA1c levels on blood glucose and lipids parameters and dyslipidaemia (defined by lipid and apolipoprotein ratios).

Preliminary results from this study indicate some unfavourable interactions of BMI and WC with the glucose and individual lipid profiles of diabetic patients during both fasting and postprandial states. Though the gender difference in our population has a limited effect on major metabolic changes, the increase in lipid and apolipoprotein levels is more pronounced with BMI and WC during the postprandial state. Our finding shows that apo B levels exceed the optimal target of $\sim$0.9 g/L (13) and are strongly influenced by BMI and WC in both genders, thereby indicating a high cardiometabolic risk in our patients. However, in addition to contributions of obesity and body fat distribution to the development of pre-diabetes and type 2 diabetes co-morbidities, obesity promotes alterations in other intermediate risks in relation to dyslipidaemia, glucose intolerance, the inflammatory state, as additional unknown mechanisms may vary between the genders (20, 21). Gender differences regarding the effect of overweight/obesity on metabolic disorder is likely to result from the influence of several factors, such as delayed dietary fat clearance, which results from abdominal adipose tissue accumulation in men, along with a concomitant decrease in the postprandial metabolism of fatty acids and a decreased ability to store lipids in subcutaneous adipose tissue (22).

The risk of vascular disease is two- to four-fold greater in adults with diabetes than among non-diabetic individuals (23). However, association between diabetes and risk factors for CVD, such as dyslipidaemia (previously called hyperlipaemia), hypertension, and hyperinsulinaemia, bring out the necessity of an aggressive management (24). Therefore, tables of cardiovascular risk (e.g. the Framingham score or the EuroSCORE) (25, 26) and international recommendations (e.g. recommendations of the National Cholesterol Education Program – NCEP, 2001) (27), centred on the classic lipid profile, were developed to validate the effectiveness of lipid-lowering therapies. But important limitations of these parameters in cardiovascular risk prediction have been demonstrated (28). There is now more evidence from cohort and meta-analysis studies suggesting that lipid ratios (TC/HDL-c, TG/HDL-c, and LDL-c/HDL-c) have higher association with CVD than with individual lipids (29–32). On the contrary, prospective risk studies suggest that the use of apo B/apo A-I ratio may be a promising and convenient marker of risk of CVD [e.g. AMORIS study (13), INTERHEART study (14), EPIC-Norfolk study (33), and ULSAM study (34)].

The apo A-I, apo B, and apo B/apo A-I ratios have many advantages that surpass their use compared with normal lipid parameters and their ratios in predicting CVD. Apo B reflects the atherogenic side and apo A-I indicates the anti-atherogenic side. Hence, the apo B/apo A-I ratio reflects the risk of cardiovascular events (13). Another important feature is that apolipoproteins concentrations are not affected by meals and are slightly influenced by biological variables, unlike the ordinary lipid parameters, which fluctuate widely depending on food intake. Therefore, measurement of apolipoproteins does not require fasting blood samples (12, 13, 35, 36). In clinical practice, apolipoproteins A-I and B may be measured directly in plasma without noticeable interference with high triglyceride levels using internationally standardised methods that are accurate and precise (12, 37).

The results of this study clearly show, for both genders, that BMI, WC, and impaired glycaemia (defined by HbA1c) were proportionally related with the degree of dyslipidaemia during both fasting and postprandial states. During the postprandial stage, the apolipoproteins ratio (apo B/apo A-I) was more correlated with TC/HDL-c ($r^2 = 0.321$, $p < 0.01$, $F = 111.543$) and LDL-c/HDL-c ($r^2 = 0.300$, $p < 0.001$, $F = 103.317$) and lightly correlated with TG/HDL-c ($r^2 = 0.142$, $p < 0.001$, $F = 39.756$). Similar results have been presented in other studies (9, 10, 38–42).

Although the vast majority of patients with established type 2 diabetes should be considered at high short-term risk, the association of diabetes with other risk factors such as overall and/or visceral obesity and blood glucose alterations should lead to intensive monitoring and management of this situation. Our findings indicate that all TC/HDL-c and LDL-c/HDL-c ratios were beyond the values of the therapeutic target identified by most authors (4.0 for TC/HDL-c and 2.5 for LDL-c/HDL-c) (43, 44), which reflects a high risk of CVD in all patients. Values for

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the apo B/apo A-I ratio during therapy should preferably be reduced to <0.7 or even lower in patients with great risk (45). After adapting our data with the risk ranges of AMORIS and INTERHEART studies (13, 14), we found that women are more prone to cardiovascular risk (myocardial infarction) than men in terms of increased apo B/apo A1 ratio.

One limitation of the present study is the short postprandial time (3–4 h), because changes may affect lipid profiles beyond that time. The effect of age differences and genetic variations between the two genders is another limitation; variations in genes such as those encoding apolipoproteins A1 and B may directly affect postprandial lipaemia due to their involvement in lipid metabolism. Finally, information about what each participant had eaten for breakfast before blood sampling was based on self-declaration of the patients themselves.

In conclusion, most patients with type 2 diabetes, whether male or female, have dyslipidaemia to varying degrees. Dyslipidaemia, one of the CVD risk factors, becomes more severe with higher BMI and/or WC and with increased levels of HbA1c. BMI coupled with WC is more predictive of cardiovascular risk than each body parameter separately.

Compared with individual lipid parameters, the changes in lipid ratios could reflect impaired lipid metabolism at earlier stages. However, lipid ratios are often influenced by food intake, which could make interpretation of the results more difficult depending on the fasting or post-prandial state. There are now a number of user-friendly reasons for replacing traditional lipids and their ratios with measures of apo B and apo A-I and their ratio in clinical practice. As apolipoproteins can be analysed in non-fasting samples, this is of great practical advantage for patients and doctors. In addition, it is very helpful to use a single ratio for risk prediction instead of referring to a larger number of lipid ratios.

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References

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. 2010; 87: 4–14.
2. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes. 2008; 32: 1431–7.
3. Snijder MB, van Dam RM, Visser M, Seidell JC. What aspects of body fat are particularly hazardous and how do we measure them? Int J Epidemiol. 2006; 35: 83–92.
4. Resnick HE, Valsania P, Halter JB, Lin X. Differential effect of BMI on diabetes risk among black and white Americans. Diabetes Care. 1998; 21: 1828–35.
5. Haffner SM, Mitchell BD, Hazuda HP, Stern MP. Greater influence of central distribution of adipose tissue on incidence of non-insulin-dependent diabetes in women than men. Am J Clin Nutr. 1991; 53: 1312–17.
6. Chan JM, Rimm EB, Colditz CA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care. 1994; 17: 961–9.
7. Boyko EJ, Fujimoto WY, Leonetti DL, Newell-Morris L. Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans. Diabetes Care. 2000; 23: 465–71.
8. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesityrelated health risk. Am J Clin Nutr. 2004; 79: 379–84.
9. Khan HA, Sobki SH, Khan SA. Association between glycaemic control and serum lipids profile in type 2 diabetic patients: HbA1c predicts dyslipidaemia. Clin Exp Med. 2007; 7: 24–9.
10. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. Am J Clin Nutr. 2005; 81: 555–63.
11. Bergmeyer HU. Methods of enzymatic analysis. New York: Verlag Chemie; 1974.
12. Tietz NW. Fundamentals of clinical chemistry, 3rd ed. Philadelphia, PA: W. B. Saunders; 1987.
13. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS Study): a prospective study. Lancet. 2001; 358: 2026–33.
14. Yusuf S, Hawken S, Öunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364: 937–52.
15. James WP. The epidemiology of obesity: the size of the problem. J Intern Med. 2008; 263: 937–52.
16. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. J Clin Endocrinol Metabol. 2004; 89: 2595–600.
17. Yusuf S, Hawken S, Ounpuu S, Bautista L, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet. 2005; 366: 1640–9.
18. Canoy D, Boekholdt SM, Wareham N, Luben R, Welch A, Bingham S, et al. Body fat distribution and risk of coronary heart disease in men and women in the European prospective investigation into cancer and nutrition in Norfolk cohort: a population-based prospective study. Circulation. 2007; 116: 2933–43.
19. Elley CR, Kenealy T, Robinson E, Drury PL. Glycated haemoglobin and cardiovascular outcomes in people with Type 2 diabetes: a large prospective cohort study. Diabet Med. 2008; 25: 1295–301.
20. Bastiena M, Poiriera P, Lemieuxa I, Despré JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis. 2014; 56: 369–81.
21. Wormser D, Kaptoge S, Angelantonio ED, Wood AM, Pennells L, Thompson A, et al. Separate and combined associations body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet. 2011; 377: 1085–95.
22. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, et al. Gender difference in postprandial lipemia: importance of visceral adipose tissue accumulation. Arterioscler Thromb Vasc Biol. 1999; 19: 2448–55.
23. Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, et al. The burden of mortality attributable to diabetes:

realistic estimates for the year 2000. Diabetes Care. 2005; 28: 2130–5.
24. Goldberg IJ. Diabetic dyslipidemia: causes and consequences. J Clin Endo Metab. 2001; 8: 965–71.
25. Lloyd-Jones DM, Wilson PWF, Larson MG, Beiser A, Leip EP, D'Agostino RB, et al. Framingham risk score and prediction of lifetime risk for coronary heart disease. Am J Cardiol. 2004; 94: 20–4.
26. Nashef SA, Roques F, Michel P, Gauducheau E, Lemeshow S, Salamon R. European system for cardiac operative risk evaluation (EuroSCORE). Eur J Cardiothorac Surg. 1999; 16: 9–13.
27. Alexander CM, Landsman PB, Teutsch SM, Haffner SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes. 2003; 52: 1210–14.
28. Gotto AM, Jr., Whitney E, Stein EA, Shapiro DR, Clearfield M, Weis S, et al. Relation between baseline and ontreatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/Tex CAPS). Circulation. 2000; 101: 477–84.
29. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA. 2007; 298: 776–85.
30. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths. Lancet. 2007; 370: 1829–39.
31. Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, et al. 2009 Canadian cardiovascular society/ Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult – 2009 recommendations. Can J Cardiol 2009; 25: 567–79.
32. Zhao W, Gong W, Wu1 N, Li Y, Ye K, Lu B, et al. Association of lipid profiles and the ratios with arterial stiffness in middle-aged and elderly Chinese. Lipids Health Dis. 2014; 13: 2–6.
33. Van der Steeg WA, Boekholdt SM, Stein EA, El-Harchaoui K, Stroes ESJ, Sandhu MS, et al. Role of the apolipoprotein B-apolipoprotein A-I ratio in cardiovascular risk assessment: a case-control analysis in EPIC-Norfolk. Ann Intern Med. 2007; 146: 640–8.
34. Dunder K, Lind L, Zethelius B, Berglund L, Lithell H. Evaluation of a scoring scheme, including proinsulin and the apolipoprotein B/apolipoprotein A1 ratio, for the risk of acute coronary events in middle-aged men: Uppsala Longitudinal Study of Adult Men (ULSAM). Am Heart J. 2004; 148: 596–601.
35. Tamang HK, Timilsina U, Singh KP, Shrestha S, Raman RK, Panta P, et al. Apo B/Apo A-I ratio is statistically a better predictor of cardiovascular disease (CVD) than conventional lipid profile: a study from Kathmandu Valley, Nepal. J Clin Diagn Res. 2014; 8: 34–6.
36. Shilpasree AS, Sahukar S, Murthy J, Kumar K. A study of serum apolipoprotein A1, apolipoprotein B and lipid profile in stroke. J Clin Diagn Res. 2013; 7: 1303–6.
37. Marcovina S, Packard J. Measurement and meaning of apolipoprotein AI and apolipoprotein B plasma levels. J Intern Med. 2006; 259: 437–46.
38. Hou X, Lu J, Weng J, Ji L, Shan Z, Liu J, et al. Impact of waist circumference and body mass index on risk of cardiometabolic disorder and cardiovascular disease in Chinese adults: a national diabetes and metabolic disorders survey. PLoS One. 2013; 8: 1.
39. Taskinen MR, Barter PJ, Ehnholm C, Sullivan DR, Mann K, Simes J, et al. Ability of traditional lipid ratios and apolipoprotein ratios to predict cardiovascular risk in people with type 2 diabetes. Diabetologia. 2010; 53: 1846–55.
40. Yan A, Liu Y, Huang H. Association of glycosylated hemoglobin level with lipid ratio and individual lipids in type 2 diabetic patients. Asian Pac J Trop Med. 2012; 5: 469–71.
41. Belfki H, Ben Ali S, Bougatef S, Ben Ahmed D, Haddad N, Jmal A, et al. The apolipoprotein B/apolipoprotein A 1 ratio in relation to metabolic syndrome and its components in a sample of the Tunisian population. Exp Mol Pathol. 2011; 91: 622–5.
42. Salazar MR, Carbajal HA, Espeche WG, Aizpurúa M, Maciel PM, Reaven GM. Identification of cardiometabolic risk: visceral adiposity index versus triglyceride/HDL cholesterol ratio. Am J Med. 2014; 127: 152–7.
43. Leiter LA, Genest J, Harris SB, Lewis G, McPherson R, Steiner G, et al. Dyslipidemia in adults with diabetes. Can J Diabetes. 2006; 30: 230–40.
44. Pereira T. Dyslipidemia and cardiovascular risk: lipid ratios as risk factors for cardiovascular disease. In: Kelishadi R (Ed.), editor. Dyslipidemia – from prevention to treatment. Rijeka: InTech; 2012. p. 279–302.
45. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, et al. Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA. 2006; 295: 1556–65.

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Listof publications and communications carried out as part of the thesis

Publications and abstracts proceeding

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Mustapha DIAF, Boumediene Meghit KHALED, HadjHabib HOUARI, Slimane BELBRAOUET. Effect of gender and body weight on postprandial glucose and lipid metabolism in adults with type 2 diabetes (Original Article n° 2378).Journal of Nepal Medical Association (JNMA).Accepted on 2014-10-31.(In press).

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Communications

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DIAF M, KHALED MB. Lipémie postprandiale, lipémie à jeun et risques cardiovasculaires chez les femmes diabétiques. Les 1ières Journées Scientifiques sur les Sciences de la Nature et de la Vie. 22-23 Janvier 2014, Université Djillali LIABES de Sidi-Bel-Abbès.

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