JULIANO ALVES PEREIRA

AVALIAÇÃO DAS MODIFICAÇÕES METABÓLICAS DURANTE E APÓS O EMAGRECIMENTO EM PACIENTES PORTADORES DE OBESIDADE CLASSE III, SUBMETIDOS À CIRURGIA DE GASTROPLASTIA VERTICAL COM BANDAGEM E DERIVAÇÃO GASTRO-JEJUNAL.

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"A coisa mais bonita que podemos experimentar é o mistério,
que é a fonte de toda a verdadeira arte e ciência."

Albert Einstein

Ác Úrico	Ácido Úrico
ASC	Área sob a curva
BIA	Bioimpedância Elétrica
Cl Ins	Clearence de Insulina
Clamp	Clamp euglicêmico-hiperinsulinêmico
CO_2	Gás carbônico
O_2	Oxigênio
CoA	Coenzima A
Cr	Creatinina
d	Dia
DM tipo2	Diabetes Mellitus Tipo 2
f	Feminino
FC	Freqüência Cardíaca
FCM	Faculdade de Ciências Médicas
GE	Gasto Energético
HAS	Hipertensão Arterial Sistêmica
HC	Hospital das Clínicas
HOMA	*Homeostatic Model Assesment*
IDR	*Insulin Delivery Rate* (Taxa de liberação de insulina)
IMC	Índice de Massa Corpórea

JNC	*Joint National Comitte*
K	Potássio
kcal	Quilocalorias
kg	Quilograma
M	Índice de Sensibilidade à Insulina
m	Masculino
ml	Mililitro
MM	Massa Magra
mm	Milímetro
mmHg	Milímetros de Mercúrio
Na	Sódio
NHI	*National Institute Of Health*
ns	Não significativo (Relação Estatística)
O_2	Oxigênio
ºC	Graus Celsius
OGTT	*Oral Glucose Tolerance Test*)
PA	Pressão Arterial
PAD	Pressão Arterial Diastólica
PAS	Pressão Arterial Sistólica
QR	Coeficiente Respiratório não Protéico
SS	*Steady State* (período de estabilidade no *clamp*)
TIG	Taxa de infusão de glicose

UI	Unidades Internacionais
UM	Unidade Metabólica
UNICAMP	Universidade Estadual de Campinas
WHO	*World Health Organization*

RESUMO

A obesidade é uma doença com alta prevalência nos países ocidentalizados e, do ponto de vista metabólico, é caracterizada por resistência a várias ações da insulina e hiperinsulinemia. A hiperinsulinemia em obesos parece relacionada à hipersecreção hormonal compensatória à reduzida ação insulínica.

A significativa redução de peso observada em pacientes submetidos à cirurgia bariátrica permite avaliar o impacto do emagrecimento, em várias alterações patológicas encontradas em pacientes obesos. Os mecanismos, o grau de emagrecimento necessário e quais das alterações se modificam não estão totalmente esclarecidos.

Treze pacientes obesos classe III, 4 homens e 9 mulheres, IMC = 56,3 ± 2,7 $kg.m^{-2}$ foram estudados antes, durante e após a estabilização do peso e os resultados foram comparados a um grupo de indivíduos controle com peso normal (5M/8F; IMC = 22,4 ± 0,5 $kg.m^{-2}$). IMC final dos obesos - 34.7 ± 2.1 kg/m^2 .

Após um primeiro estudo metabólico, os obesos foram submetidos à gastroplastia vertical com bandagem e derivação gastrojejunal (técnica de Capella). Foram reavaliados depois do emagrecimento em torno de 20% do peso inicial e depois de estabilização do peso corporal (~-40%). A composição corporal avaliada por impedância bioelétrica melhorou, verificando-se uma redução maior de massa magra que de massa adiposa.

Os pacientes obesos apresentaram níveis plasmáticos altos de insulina em jejum (6 vezes maior que a do grupo controle) e durante o teste de tolerância oral à glicose. Após o emagrecimento, a concentração de insulina em jejum foi semelhante à encontrada no grupo de indivíduos magros. A hiperinsulinemia era decorrente de hipersecreção, uma vez que, os níveis de peptídeo C circulantes também eram altos e o clearance de insulina, avaliado durante *clamp* euglicêmico hiperinsulinêmico, discretamente aumentado, comparado ao dos magros.

A sensibilidade à insulina foi avaliada através do clamp euglicêmico hiperinsulinêmico (infusão de insulina – 40 mU/m^2.min). Antes da cirurgia, era reduzida em obesos (M – obesos = 19.7 ± 1.5 *vs*. magros = 51.5 ± 2.4 umol/min.kg MM; $p<0.001$) e aumentou significativamente após o final da fase de emagrecimento (35,5 ± 3,7 umol/min.kg MM;

$p<0.05$ *vs*. pré-cirurgia), mas ainda foi menor que aquela medida no grupo de indivíduos magros ($p<0.0001$).

O uso associado da calorimetria indireta permitiu verificar que tanto a via oxidativa quanto a não oxidativa foram pouco estimuladas pela infusão de insulina nos obesos. Depois do emagrecimento, apenas a resposta da via não oxidativa melhorou de maneira significativa, atingindo valores semelhantes aos observados no grupo controle. A oxidação protéica era menor que a dos magros e aumentou de forma similar durante o *clamp*. A oxidação lipídica em jejum, ao contrário, era maior nos obesos comparados aos magros e não foi inibida pela insulina no *clamp* realizado antes da cirurgia. Depois do emagrecimento, permaneceu alta em jejum e foi inibida discretamente durante o clamp. Estas repostas foram significativamente diferentes das observadas nos magros.

No grupo controle, ocorreu uma inibição da secreção endógena de insulina, atribuída à própria insulina e avaliada pelos níveis plasmáticos de peptídeo-C. Esta auto-inibição da insulina estava diminuída nos obesos e foi totalmente recuperada após o emagrecimento.

Em conclusão, este estudo permitiu confirmar várias alterações metabólicas relacionadas à secreção ou à ação da insulina em obesos. Além disto, demonstrou a falta da auto-inibição da secreção de insulina e sua recuperação pelo emagrecimento rápido e importante após cirurgia bariátrica. Demonstrou ainda, que a via metabólica que melhor respondeu ao emagrecimento foi a não oxidativa da glicose. Os efeitos do emagrecimento na sensibilidade à insulina e na secreção de insulina foram desproporcionais e ocorreram em tempos distintos da evolução pós-cirúrgica.

ABSTRACT

Obesity has a high prevalence and an increasing incidence, being considered epidemic. It is characterized by hyperinsulinemia and insulin resistance, important early metabolic changes in the obese patient. The first has been reported as an independent cardiovascular risk factor. The mechanisms underlying hyperinsulinemia are not clear and it has been associated to a compensatory beta cell mechanism for insulin resistance. The pos-hepatic insulin delivery rate is a result of the rate of insulin secretion and of its metabolic clearance rate. The main sites of insulin resistance in the obese patient are the liver and the muscle. Both insulin action pathways, oxidative and non oxidative glucose utilization - NOGD, seem to be affected.

These metabolic changes are more frequent in class III obese patients, responsible, in part, for the high mortality. Weight reduction improves metabolic and cardiovascular parameters, although it is not clear which amount of weight loss is necessary, neither which insulin effects are modified by this procedure. Bariatric surgery has been report as an efficient tool for the weight, reduction and maintain.

A group of 13 class III obese patients (4M/9F; BMI=56.3±2.7 $kg.m^{-2}$) and 13 lean subjects (5M/8F; BMI=22.4±0.5 $kg.m^{-2}$) were submitted to a metabolic evaluation including euglycemic hyperinsulinemic clamp, oral glucose tolerance test (OGTT) and indirect calorimetry. The obese patients were submitted to a bariatric surgery (Capella's technique) and the metabolic studies repeated after ~ 20 % of weight loss relative to their initial body weight (study II) and after weight stabilization (study III – BMI=34.7±02.1 $kg.m^{-2}$).

Insulin secretion during fasting state and under insulin infusion during the euglycemic clamp was assessed using the C-peptide plasma measurements. It was higher in the obese in the first study (6 folders than control) and the decrease, after weight loss, was statistically significant even in the second study, with further reduction in the third study. The hyperinsulinemia was a result of insulin hyper-secretion since pos-hepatic metabolic clearance rate during the clamp was similar to that of the control group and did not change after weight loss.

The insulin sensitivity was significantly impaired in the obese (study I: 19.7 ± 1.5 *vs*. 51.5 ± 2.4 umol/min.kgFFM; p< 0.0001) and it increased significantly after weight loss (35.5 ± 3.7 umol/min.kgFFM; p< 0.05 *vs*. CT and study I), but it was still lower than that of lean group (p<0.0001).

The insulin stimulated glucose oxidation was lower in the obese group compared to lean and improved slightly after weight loss but it was still lower than that of the former group. The non oxidative glucose disposal was reduced in the obese group and after weight loss it improved significantly reaching levels similar to those of the lean group. Fasting protein oxidation and suppression of protein oxidation by insulin infusion were similar between lean and obese and they were not influenced significantly after weight reduction. The higher fasting lipid oxidation in obese did not change by weight reduction. The insulin infusion did not suppress the lipid oxidation in the first study, but after weight reduction a slight suppression, similar to that of the control group was observed.

Insulin infusion under euglycemic conditions at steady state inhibited its own secretion in lean subjects but this insulin action was blunted in the obese group. After weight loss, inhibition of its own secretion was completely reversed to values very similar to those observed in lean controls (about 40% relative to fasting values).

In summary, the rapid weight loss in obese class III, after surgery, induced a normalization of the insulin secretion and only an improvement of the insulin sensitivity, almost entirely dependent of glucose storage. The high lipid oxidation did not change with weight loss. The changes in insulin secretion and sensitivity induced by weight loss displayed different degrees and time courses.

1. INTRODUÇÃO

1.1. OBESIDADE

A obesidade (do latin *obesus*, *ob*= muito *edere*= comer) pode ser definida como o aumento da quantidade de gordura corporal e, atualmente, é considerada uma patologia de prevalência crescente e de caráter epidêmico (ROSENBAUM et al., 1997). Representa, para a sociedade moderna, uma grande preocupação de saúde pública (BRAY, 1987). Interações entre fatores culturais, psicológicos e sociais induzem uma crescente susceptibilidade à obesidade humana, indicando ser esta uma patologia complexa (HILL e PETERS,1998; FRIDMAN e BROWNELL, 1995). O aumento do peso envolve vários mecanismos (MARYLAND, 1985), pois, além do desequilíbrio energético, a obesidade é associada a distúrbios no metabolismo dos lipídios e da glicose que predispõe ao diabetes tipo 2. As doenças cardiovasculares, em conjunto com o diabetes tipo 2, respondem pela maioria das complicações e por importante fração da mortalidade associada à obesidade.

O Índice de Massa Corporal (IMC) obtido pela relação entre peso e altura (kg/m^2) tem sido usado para definir clinicamente a obesidade (GARROW e WEBSTER, 1985). A Organização Mundial de Saúde (WHO, 1989), assim como o *National Institute of Health*, classificam pessoas com IMC maior que 25, como pessoas portadoras de sobrepeso e, com IMC maior que 30, como obesas (PI-SUNYER, 1998).

Estudos realizados por VAGUE (1956) mostraram que os efeitos adversos da obesidade estavam relacionados com o padrão de distribuição central da gordura, localizada na parte superior do corpo, característica do homem. A gordura abdominal (tipo andróide) constitui um fator de risco específico para a saúde (JENSEN, 1997), uma vez que tem sido associada à morbidade e mortalidade por doenças cardiovasculares, diabetes tipo 2 e dislipidemias (DESPRES et al., 1989). Dois importantes estudos, o *Quebec Family Study* (PERUSSE et al., 1996) e o *HERITAGE Family Study* (RICE et al., 1997) mostraram que a obesidade visceral, assim como a obesidade geral, é uma característica hereditária em quase metade dos pacientes avaliados (RICE et al., 1996).

A obesidade tem sido considerada um fator de risco independente para doenças cardiovasculares (HUBERT et al., 1983). Diversos estudos demonstraram maiores concentrações de colesterol plasmático total e LDL, triglicerídeos e concentrações menores

de HDL-colesterol (LAMARCHE et al.,1993), em indivíduos obesos, em relação àqueles com peso normal, o que contribui para o risco aterogênico relacionado à obesidade (ANDERSON et al., 1987).

As complicações respiratórias geralmente são subestimadas, apesar de serem comuns entre os obesos. Diminuição da função pulmonar, com hipóxia e hipoventilação pulmonar é freqüentemente encontrada nesta população. A apnéia obstrutiva do sono chega a atingir 24% e 9%, em homens e mulheres com sobrepeso (YOUNG et al., 1993) (RICHMAN et al., 1994), principalmente naqueles com excesso de gordura abdominal (SHINOHARA, 1997).

Osteoartrites, gota e hiperuricemia também estão nitidamente associadas à obesidade, bem como a sobrecarga ao sistema ósteo-articular e problemas cutâneos (DE GENNES, 1993). Alguns estudos sobre a mortalidade por alguns tipos de cânceres têm evidenciado que os aspectos dietéticos e endócrinos da obesidade, entre outros, constituem fatores de risco para o desenvolvimento de câncer de próstata e coloretal, nos homens, (GIOVANNUCCI et al., 1995) e de endométrio, útero, cérvix, ovários, vesícula e mama, nas mulheres (FOLSOM et al, 1996).

Apesar de todas as complicações relacionadas à obesidade, o sucesso terapêutico permanece difícil de ser obtido, principalmente nos pacientes que apresentam importante excesso de peso (MARTIN et al., 1995). O emagrecimento e a manutenção do peso, alcançado através de dietas associadas ou não a medicamentos a atividade física, ocorre apenas em um pequeno número de casos (WARDLE, 1996). A situação se torna ainda mais crítica, quando são considerados os grandes obesos ou obesos severos (GARRIDO, 1998). Estes indivíduos são classificados como obesos classe III, segundo os critérios propostos pela Organização Mundial de Saúde (WHO, 1989), apresentam o Índice de Massa Corporal, (IMC) ≥ 40 kg/m^2 e têm um risco muito alto para o desenvolvimento de co-morbidades (SJÖSTROM, 1992a). MARTIN et al. (1995) estimaram que a prevalência de obesos classe III seja de 2 e 6 % entre homens e mulheres norte-americanos, respectivamente.

Um estudo realizado com obesos classe III, na faixa etária de 25 a 34 anos, evidenciou mortalidade geral 12 vezes maior que em pessoas de peso normal (DRENNICK et al., 1980). A grande incidência de doença arterial coronariana, hipertensão arterial sistêmica, disfunção cardíaca (SJÖSTROM, 1992b), diabetes tipo 2 e apnéia obstrutiva do sono (RAJALA et al., 1991) contribuem para a alta mortalidade neste grupo. Artropatias, osteoartrite degenerativa, colelitíase, hérnias, alterações menstruais e infertilidade, entre outras co-morbidades, são muito comuns nestes pacientes (WINIARSKY et al.,1998; MARTIN et al.,1998).

Qualidade de vida insatisfatória, ocorrência de distúrbios psicológicos e sociais, alta prevalência de patologias associadas e altas taxas de mortalidade (SJÖSTROM 1992b) levaram os autores a reconhecer este tipo de obesidade como obesidade mórbida, embora muitos considerem este termo inadequado (GARRIDO,1998).

1.2. OBESIDADE E ALTERAÇÕES METABÓLICAS:

A resistência à insulina é definida por FERRANNINI (1999), como sendo uma alteração metabólica relacionada à diminuição de uma ou mais respostas biológicas à ação da insulina. Em conjunto, a diminuição da secreção de insulina (basal e estimulada pela glicose), a aumentada produção hepática de glicose e a resistência periférica à insulina (MOLLER e FLIER, 1991) são consideradas as principais alterações metabólicas, presentes no indivíduo diabético tipo 2 (DEFRONZO, 1997).

Estudo realizado em um grupo de índios norte-americanos *Pima,* caracterizado por alta incidência de diabetes tipo 2, mostrou que a transição de tolerância normal para intolerância à glicose estava associada apenas com o aumento de resistência à insulina (LILLIOJA et al., 1998). No entanto, alterações na secreção de insulina foram observadas após o início do quadro típico do diabetes tipo 2. Deste modo, resistência à insulina foi a primeira anormalidade metabólica detectada como causa de intolerância à glicose. Provavelmente seja relacionada com causas genéticas, por se apresentar como uma característica familiar nesta população (LILLOJA e BOGARDUS, 1987). BOGARDUS,

et al. (1984a) encontraram uma relação inversa entre o IMC e a sensibilidade à insulina na mesma população. Por sua vez, a diminuição da sensibilidade à insulina está associada com o aumento da morbidade e mortalidade, independentemente de outros fatores de risco (MANSON et al., 1990).

A resistência à insulina também tem sido observada em muitos pacientes com hipertensão arterial (FERRANNINI et al., 1987) e é considerada um fator de risco para infarto agudo do miocárdio, em pacientes jovens, com menos de 40 anos (CAVALLO-PERIN et al., 2001). Vários pesquisadores têm apontado a resistência à insulina como a alteração primária que poderia levar a uma combinação de diabetes tipo 2, hipertensão arterial, dislipidemia, hiperuricemia, e associada à obesidade central (padrão andróide de distribuição de gordura corporal) (DEFRONZO e FERRANNINI, 1991; EVANS et al., 1984). Estas alterações metabólicas, dependendo da susceptibilidade genética individual (SORENSEN, 1995), têm sido responsabilizadas pela aceleração do processo aterogênico (LAAKSO, et al. 1991) e pelo aumento das doenças cardiovasculares (BRESSLER et al., 1996), principais responsáveis pela alta mortalidade precoce em pacientes diabéticos (FERRANNINI, 1999). Esta complexa interação patológica tem sido referida como Síndrome X (REAVEN, 1988; 1994), Síndrome da Resistência à Insulina (DEFRONZO e FERRANNINI, 1991) ou Síndrome Metabólica (MEIGS, 2000; MOLLER e FLIER, 1991). Estudo recente nos EUA, sugere que, aproximadamente, 50 milhões de residentes nos Estados Unidos apresentem a síndrome metabólica (FORD et al., 2002), definida de acordo com os critérios do National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III - NIH Publication 01-3670, 2001).

A prevalência de resistência à insulina e hiperinsulinemia entre os grandes obesos, obesos classe III, não é conhecida. Vários estudos que acompanharam estes obesos não diabéticos, antes de serem submetidos à cirurgia, sugeriram uma alta prevalência de ambas (JIMENEZ et al., 1986; LETIEXHE et al.,1995).

Avaliações da sensibilidade à insulina, pelo método *clamp* euglicêmico hiperinsulinêmico, em humanos obesos, evidenciaram que a captação de glicose estimulada pela insulina foi menor quando comparada à de pessoas magras (BONADONNA et al.

1990; MUSCELLI et al. 1997, 2001). Além disto, técnicas capazes de avaliar a distribuição de gordura corporal (THOMAS et al 1998) mostram uma relação direta entre resistência à insulina e o acúmulo de gordura intra-abdominal, também conhecida como gordura visceral. (BJÖRNTORP, 1987; 1990; EVANS et al., 1984; BONORA, 2000). As razões que podem levar a esta associação ainda não são claras (FRAYN, 2000), mas vários estudos prospectivos confirmam que a gordura localizada na parte superior do corpo, padrão andróide, está associada a várias alterações metabólicas (FONTBONNE e ESCHWÈGE, 1991; DUNCAN et al., 1995), incluindo aquelas relacionadas ao metabolismo da glicose (BONORA et al., 1992).

A hiperinsulinemia é uma alteração metabólica freqüente na obesidade (BONADONNA et al., 1990; OLEFSKY et al., 1982), também encontrada em pacientes diabéticos tipo 2 (POLONSKY, 2000). O nível de insulina circulante é o resultado da taxa de secreção insulínica e de seu *clearance* metabólico. Este foi considerado normal, nos obesos avaliados por MUSCELLI et al. (1997) e por POLONSKY et al. (1988). Entretanto, diferenças entre o *clearance* de pacientes obesos e magros foram relatadas (MEISTAS et al., 1983). Desta forma, a hiperinsulinemia provavelmente se deve à hipersecreção desse hormônio, amplamente demonstrada na obesidade (POLONSKY et al. 1988 a,b ; WILLER et al., 1992; FERRANNINI et al.,1997).

A secreção de insulina, por sua vez, é determinada por complexas influências estimulatórias e inibitórias, sobre a célula beta (BRATUSCH-MARRAIN e WALDHÄUSL, 1985). Um dos estímulos para a maior secreção é a resistência tecidual à insulina (JONES C.H.O. et al., 2000). Entre as influências inibitórias, alguns estudos sugerem a existência de um *feedback* negativo, direto ou indireto, da insulina circulante sobre a secreção da própria célula beta (DEFRONZO et al., 1981). LILJENQUIST et al. (1978) demonstraram inibição da secreção de insulina, em humanos normais, mas outros não obtiveram este resultado (PEIRIS et al., 1993). Em pacientes obesos, juntamente à resistência generalizada à insulina, pode haver uma resistência específica na célula beta, responsável pela falha no mecanismo inibitório. Apesar de ainda controverso, estudos de CAVALLO-PERIN et al. (1993) e de HIROTA et al. (1987) demonstraram uma falha no *feedback* de insulina, enquanto outros observaram uma inibição similar à de indivíduos magros (ELAHI et al., 1982).

CAVALLO-PERIN et al. (1988) encontraram uma menor capacidade de auto-inibição da secreção de insulina, em pacientes portadores da doença de Graves, uma patologia diretamente relacionada ao aumento da sensibilidade à insulina. Entretanto, estudos em diabéticos mostraram resultados discordantes (HIROTA et al., 1987; BAYNES et al., 1991).

A inibição da secreção de insulina estimulada por glicose, a partir da somatostatina, mantém-se inalterada nos obesos (BONORA et al., 1990; SCHUSDZIARRA et al., 1985), o que sugere um defeito específico no *feedback* de insulina e insulino-resistência.

Em pessoas obesas, a alteração da sensibilidade à insulina pode ocorrer também no fígado, com inadequada supressão da produção hepática de glicose (OAKES et al., 1997b). A falta de inibição da produção endógena (principalmente hepática) de glicose tem sido a grande responsável pela hiperglicemia de jejum, observada em pacientes obesos (BOGARDUS et al., 1984 b; FIRTH e RIZZA, 1987). Alguns trabalhos têm mostrado (DEFRONZO et al., 1989) uma correlação linear positiva entre a hiperglicemia plasmática de jejum e a taxa de produção hepática de glicose, em pacientes diabéticos após a alimentação.

1.3. OBESIDADE, INSULINA E UTILIZAÇÃO DE SUBSTRATOS:

A oxidação de glicose e a utilização não oxidativa são as duas principais vias de utilização intracelular da glicose (DEFRONZO, 1997). O total de glicose oxidada é obtido através da calorimetria indireta e a quantidade de glicose não oxidada corresponde à diferença entre o total de glicose infundida durante o *clamp* e a glicose oxidada (FERRANNINI, 1988). Estudos de THIEBAUD et al. (1982) mostraram que, em concentrações baixas de insulina plasmática, a quantidade de glicose utilizada nas duas vias é semelhante, mas em concentrações crescentes de insulina, a utilização não oxidativa é maior. Nesta via não oxidativa, a quase totalidade da glicose é usada para a formação de glicogênio (YOUNG et al.1988), já que apenas uma pequena parte é captada pelo músculo

e transformada em lactato (NATALI et al., 1990). Além das vias oxidativa e não oxidativa da glicose, uma quantidade reduzida é transformada em lipídios (MARIN et al.,1987).

Alterações na via não oxidativa da glicose são comuns em estados de resistência à insulina, como obesidade e diabetes (DEFRONZO, 1997). Em concordância, LILLIOJA et al. (1986) relataram que a diminuição da capacidade de utilização de glicose na via não oxidativa é a principal responsável pela captação total de glicose diminuída em pacientes obesos, com tolerância normal ou intolerantes à glicose. A menor capacidade da insulina, em estimular a via não oxidativa da glicose e em aumentar os depósitos de glicogênio, tem sido referida por alguns autores, como o defeito inicial no desenvolvimento da resistência à insulina relacionada à obesidade e ao diabetes (DEFRONZO, 1997).

1.4. AVALIAÇÃO DA SENSIBILIDADE À INSULINA:

A avaliação da ação insulínica oferece uma série de dificuldades do ponto de vista prático. O método considerado padrão ouro é o *clamp* euglicêmico hiperinsulinêmico, que é realizado infundindo-se insulina em dose constante pré-estabelecida (DEFRONZO et al., 1979). A glicemia, por sua vez, é mantida constante através de infusão concomitante de glicose hipertônica em velocidade variável, determinada pela avaliação da glicemia, em intervalos sucessivos de 5 a 10 minutos. Assim, o total de glicose infundida por minuto, somado à produção endógena de glicose, é uma medida da captação tissular induzida pelo hormônio, ou seja, é uma medida da ação insulínica. Freqüentemente, a produção endógena de glicose não é avaliada nos estudos de sensibilidade à insulina, devido ao alto custo e à necessidade de administrar substâncias marcadas, tais como isótopos estáveis ou radioativos. Além disto, resultados de estudos anteriores sugerem que a produção endógena de glicose é quase inteiramente suprimida durante infusão de insulina, mesmo em pacientes obesos (BONADONNA et al., 1990).

O tempo necessário para alcançar condições estáveis de estudo durante o *clamp* faz com que se estenda por, no mínimo, 2 horas. Além disto, uma infra-estrutura complexa e profissionais treinados são necessários. Como conseqüência, outros métodos têm sido

propostos nas últimas décadas. A associação entre hiperinsulinemia e resistência à insulina é um dos motivos pelos quais a primeira é utilizada como estimativa da Segunda, em estudos que incluem número grande de participantes. São, portanto, mais comuns os estudos que relatam a freqüência da hiperinsulinemia do que aqueles que medem a resistência à insulina em determinada população. Esta última, de maneira geral, é pouco conhecida.

1.5. EMAGRECIMENTO E METABOLISMO:

O emagrecimento, ao longo das últimas décadas, tem sido considerado muito importante para a redução das complicações da obesidade. Desta forma, foram utilizados métodos clínicos baseados, principalmente, em dietas hipocalóricas com diferentes composições nutricionais. Em paralelo, a atividade física é reconhecida como ponto chave para alcançar a redução do peso e das taxas de co-morbidades. O uso de medicamentos tem se difundido rapidamente e os resultados e efeitos colaterais são variáveis. Alguns estudos recentes comprovaram que o emagrecimento e a atividade física são essenciais, não apenas para a redução das complicações, como também para a prevenção de seu aparecimento, como era suposto pelas evidências clinicas (TUOMILEHTO et al., 2001).

GOLDSTEIN (1992) observou que a incidência de diabetes tipo 2 e dislipidemia diminuem significativamente com uma redução ponderal de 10%, concluindo que esta diminui os fatores de risco para doenças coronarianas, mais eficientemente que os exercícios aeróbicos. Segundo o autor, uma redução ponderal de 1 quilo aumentaria a sobrevida do paciente em 3 a 4 meses e uma redução de 10 quilos aumentaria em 35% sua expectativa de vida, principalmente daqueles que apresentam diabetes tipo 2.

Alguns trabalhos têm confirmado que os achados clínicos de melhora no metabolismo dos carboidratos, após redução de peso corpóreo, são decorrentes de modificações da sensibilidade à insulina e da hipersecreção deste peptídeo, (NISKANEN et al., 1996; SU et al., 1995).

O emagrecimento ocorrido após a cirurgia bariátrica tem sido associado à redução das co-morbidades e à melhora de vários parâmetros metabólicos e cardiovasculares (PORIES et al., 1992; SJÖSTROM et al., 1997). Entretanto, ainda existem controvérsias em relação à quantidade de redução de peso necessária para esta melhora. Em alguns estudos, houve uma completa normalização da sensibilidade à insulina em pacientes que alcançaram um IMC considerado normal, (MINGRONE et al.,1997; LETIEXHE Et al.,1995) mas, em outros, mesmo após um significativo emagrecimento, houve uma diminuição da hipersecreção de insulina (HOLTE et al., 1995), assim como uma melhora da resistência à insulina (BURSTEIN et al., 1995), mas não a normalização de ambas. Por outro lado, GRECO et al. (2002) e JIMENEZ et al. (1987) observaram uma completa normalização da sensibilidade à insulina, após uma discreta redução de peso, insuficiente para que estes pacientes deixassem de ser considerados obesos.

A quantidade de excesso de peso, a gravidade da resistência à insulina ou o método utilizado para o emagrecimento podem ser importantes para justificar estas diferenças. Portanto, avaliar a sensibilidade e a secreção de insulina, em pacientes obesos após diferentes graus de emagrecimento, pode auxiliar na compreensão dos mecanismos fisiopatológicos envolvidos.

1.6. CIRURGIA E OBESIDADE:

Dentre as possibilidades de tratamento da obesidade classe III, as que parecem proporcionar os melhores resultados, em termos de emagrecimento significativo e duradouro, são as cirurgias bariátricas (do grego, bariatric = peso) (ALVAREZ-CORDERO,1998; KOLANOWSKY, 1997; MASON et al., 1997). Tais procedimentos induzem reduções ponderais que alcançam 40 a 50% do peso inicial, em períodos curtos, de 1 a 2 anos. SJÖSTRON et al. (2000) acompanharam, por 8 anos, pacientes obesos classe III e constataram diminuição do peso de 16,3 ± 12,3 %, no grupo submetido à cirurgia, enquanto o grupo de pacientes que recebeu tratamento clínico apresentou um aumento de 0,9 ± 10,8%.

O objetivo da cirurgia é induzir e manter uma perda de peso estável e segura, suficiente para que haja redução das co-morbidades clínicas (SJÖSTRON et al., 1997; 1999), diminuição do risco de morte, melhora dos padrões metabólicos, (PORIES et al., 1992; BURSTEIN et al., 1995) melhora das funções respiratórias (SUGERMAN, et al., 1992a) e melhora da qualidade de vida. A cirurgia bariátrica está indicada para indivíduos que apresentem índice de massa corporal $\geq$ 40 kg/m^2 ou $\geq$ 35 kg/m^2, se associado a co-morbidades (NIH, 1991). O alcoolismo, doenças psiquiátricas, e idade inferior a 18 ou superior a 65 anos são algumas contra-indicações relativas (MARTIN et al.,1998; BEHRNS et al., 1993). Cirurgias bariátricas anteriores, que não tenham atingido o objetivo esperado, assim como outras cirurgias abdominais e faixa etária não devem ser, obrigatoriamente, consideradas contra-indicações (MURR et al.,1995). Pacientes não cooperativos, usuários de substâncias ativas ou dependentes de drogas e aqueles que apresentem distúrbios psiquiátricos como esquizofrenia, depressões graves e transtornos de personalidade não devem ser submetidos à cirurgia. Aspectos psicológicos e comportamentais devem ser avaliados por uma equipe multidisciplinar e o risco cirúrgico de cada paciente cuidadosamente estudado pela equipe médica. (NIH, 1991)

As primeiras cirurgias para o emagrecimento, realizadas na década de 50, tiveram como objetivo a redução da absorção dos nutrientes (PAYNE e WIND, 1969). O comprimento intestinal era reduzido através de uma anastomose entre o jejuno proximal e o íleo proximal e, assim, ocorria diminuição da superfície de absorção (MASON e ITO, 1969; SUGERMAN et al., 1992b). Apesar de promoverem redução de aproximadamente 40% do peso pré-cirúrgico, foram observadas algumas complicações nutricionais e metabólicas decorrentes da má-absorção (SMITH, et al., 1995). Insuficiência hepática (HALVERSON, et al. 1978), cirrose (KROYER e TALBERT, 1980), esteatose (LUYCKX et al., 1999), litíase renal (oxalato) (SUGERMAN et al., 1997), enterite na porção exclusa do trato alimentar, hipocalemia, e hipovitaminose (vitaminas B12 e K) foram algumas das complicações relacionadas a este tipo de cirurgia.

Na década de 60, surgiram os primeiros trabalhos restringindo a capacidade gástrica (GOMEZ, 1979). Através da gastroplastia e de alterações na parte distal do estômago, foram verificadas mudanças nos hábitos alimentares e reduções no consumo de

alimentos sólidos (PACE et al., 1993). Inicialmente, a gastroplastia foi realizada no plano horizontal e dividia o estômago em dois compartimentos de tamanhos diferentes, unidos por um pequeno orifício de passagem. MASON (1982) foi o primeiro a utilizar o plano vertical para a redução da cavidade gástrica. A exclusão de parte do estômago do trânsito digestivo também tem sido proposta para tratar a obesidade. (MASON e ITO 1969). A cirurgia original dividia o estômago em uma cavidade menor, vertical, com aproximadamente 30 ml de capacidade, próxima à cárdia e outra maior, distal, que excluía o restante do estômago e o duodeno do trânsito alimentar, mas ligadas ao intestino em Y de Roux. (GRIFFEN et al., 1977; FLICKINGER et al., 1984).

Através da utilização de grampos metálicos, foi obtida uma linha de grampeamento que se estendia da porção proximal do estomago até o ângulo de His. Associada a esta linha, foi proposta uma banda de reforço na abertura distal da nova cavidade, com o objetivo de evitar o seu alargamento e a dilatação da cavidade (SUGERMAN et al., 1989). Esta técnica passou a ser conhecida como gastroplastia vertical com bandagem e a ser utilizada por outros cirurgiões (CAPELLA e CAPELLA, 1991; CAPELLA e CAPELLA, 1996). Nos últimos anos, foi proposto o uso de um anel de contenção, para diminuir a velocidade de esvaziamento gástrico (BROLIN et al., 1994). A ingestão de alimentos hipercalóricos sob a forma líquida, a deiscência da linha de grampeamento e a estenose da anastomose gastrojejunal foram algumas complicações relacionadas a esta técnica (NIGHTENGALE et al., 1991). Alguns estudos mostraram que a perda de peso obtida ao longo de períodos maiores que dois anos foi insatisfatória (KELLUM et al., 1998). Isto levou alguns cirurgiões a abandonarem esta técnica, enquanto outros propuseram modificações na proposta inicial (JONES K.B., 2000).

Outra variação da cirurgia bariátrica é o *bypass* bilio-pancreático, descrito por SCOPINARO et al. (1979). Esta técnica utiliza o princípio da má-absorção de nutrientes, mas tem como principal mecanismo o escoamento das secreções pancreáticas e biliares para o íleo distal, a uma distância de 50 cm da junção íleo-ceco. Desta forma, os alimentos entram em contato com as secreções digestivas apenas nesta porção intestinal. Além deste procedimento, é feita uma gastrectomia distal, com propósito restritivo. Assim, a combinação de uma gastro-ileostomia, um trato biliar longo e um canal alimentar comum

curto resulta em uma significativa má-digestão e má-absorção. Estes procedimentos têm sido reportados como muito eficientes em promover e manter um intenso emagrecimento, em pacientes extremamente obesos ou super obesos (IMC≥ 50), por SCOPINARO et al. (1996). Apesar de ser uma das técnicas mais eficientes para o emagrecimento, foram observados vários efeitos colaterais tais como: má-absorção de ferro, cálcio e vitamina B12 e deficiências de vitaminas lipossolúveis, A, D, E e K (SCOPINARO et al., 1996; MURR et al., 1999). O *bypass* bilio-pancreático com duodenal *switch*, combina os efeitos de uma restrição gástrica a uma parcial má-absorção e má-digestão (MARCEAU et al., 1998). Nesta técnica, os nutrientes e as secreções digestivas se encontram a aproximadamente 100 cm da junção íleo-ceco, formando um longo canal digestivo. Apesar dos bons resultados em relação à perda de peso, o risco de subnutrição, a deficiência de vitaminas e minerais persiste (SUGERMAN et al., 1997).

A utilização de bandas gástricas ajustáveis surgiu como proposta para o emagrecimento nos últimos anos (FORSELL e HELLERS 1997). É colocada ao redor do estômago proximal, geralmente por via laparoscópica e tem a função de restringir a quantidade de alimento ingerida (O'BRIEN et al., 1999). Este procedimento, considerado interessante por alguns cirurgiões por ser pouco invasivo, tem tido resultados insatisfatórios em relação à manutenção da perda de peso em longos períodos (DOLDI et al., 2000). Complicações como erosão, sangramentos e obstruções têm sido associadas ao seu uso (NIGHTENGALE et al., 1991) e alguns estudos mostram alta incidência de refluxo gastro-esofágico (DIXON e O'BRIEN, 1999).

2. JUSTIFICATIVA E OBJETIVOS

JUSTIFICATIVA:

Embora muitos estudos prospectivos, acompanhando obesos classe III submetidos aos diferentes tipos e técnicas de cirurgias bariátricas, tenham sido realizados, uma relevante questão clínica, ainda não respondida, é qual a extensão da perda de peso suficiente para normalizar as alterações metabólicas presentes na obesidade grave.

No que se refere à resistência à insulina, não estão esclarecidas quais vias metabólicas respondem à perda de peso após o rápido emagrecimento observado em pacientes obesos submetidos à cirurgia bariátrica. A associação de calorimetria à infusão insulínica (*clamp* euglicêmico hiperinsulinêmico) permite a avaliação das vias oxidativa e não oxidativa do metabolismo dos carboidratos, bem como da oxidação lipídica e protéica.

Desta forma, a avaliação dos pacientes obesos, em etapas sucessivas de redução ponderal, utilizando os dois métodos citados, pode fornecer informações específicas quanto ao paralelismo entre perda de peso e resposta metabólica.

OBJETIVOS:

1. Estudar e comparar, entre indivíduos obesos classe III e indivíduos magros, pareados por sexo e idade, os seguintes parâmetros:

- a ação da insulina relativa ao metabolismo dos carboidratos
- a secreção de insulina: em jejum e após ingestão de glicose
- a capacidade de inibição da insulina sobre a sua própria secreção
- a ação da insulina nas vias oxidativas dos lipídios e proteínas
- a composição corporal
- o gasto metabólico basal e o coeficiente respiratório

2. Acompanhar a evolução de todas as variáveis acima, em duas fases distintas: durante o emagrecimento e após a estabilização do peso nos pacientes obesos classe III submetidos à cirurgia de gastroplastia vertical com bandagem e derivação gastrojejunal em Y de Roux (técnica de Capella).

3. Comparar todos os parâmetros descritos entre os voluntários magros e os pacientes emagrecidos pela cirurgia de Capella.

3. CAPÍTULOS

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3.5. ARTIGOS

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PAPER

Lack of insulin inhibition on insulin secretion in non-diabetic morbidly obese patients

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OBJECTIVE: Insulin inhibition of insulin secretion has been described in normal lean subjects. In this study, we examined whether this phenomenon also occurs in the morbidly obese who often have severe peripheral insulin resistance.
SUBJECTS: Twelve obese patients, normotolerant to glucose (8 F/4 M, body mass index (BMI) = 54.8 ± 2.5 kg/m^2, 39 y) and 16 lean control subjects (10 F/6 M, BMI = 22.0 ± 0.5 kg/m^2, 31 y).
DESIGN AND MEASUREMENTS: An experimental study using various parameters, including an euglycemic hyperinsulinemic clamp (280 pmol/min/m^2 of body surface), an oral glucose tolerance test (OGTT), electrical bioimpedance and indirect calorimetry.
RESULTS: The obese subjects were insulin resistant (M = 19.8 ± 1.6 *vs* 48.7 ± 2.6 μmol/min kg FFM, $P < 0.0001$) and hyperinsulinemic in the fasted state and after glucose ingestion. Fasting plasma C-peptide levels (obese 1425 ± 131 pmol/l *vs* lean 550 ± 63 pmol/l; $P < 0.0001$) decreased less during the clamp in the obese groups (−16.9 ± 6.9% *vs* −43.0 ± 5.6% relative to fasting values; $P = 0.007$). In the lean group, the C-peptide decrease during the clamp (percentage variation) was related to insulin sensitivity, M/FFM ($r = 0.56$, $P = 0.03$), even after adjustment for the clamp glucose variation.
CONCLUSION: We conclude that, in lean subjects, insulin inhibits its own secretion, and this may be related to insulin sensibility. This response is blunted in morbidly obese patients and may have a role in the pathogenesis of fasting hyperinsulinemia in these patients.
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Keywords: obesity; insulin secretion; insulin clamp; insulin resistance; C-peptide

Introduction

Obesity is a risk factor in type 2 diabetes mellitus,[1,2] hypertension and coronary heart disease.[3,4] Hyperinsulinemia and insulin resistance are important early metabolic changes in the obese.[5,6] The mechanism underlying this hyperinsulinemia is not known. Circulating insulin levels represent a balance between the rate of insulin secretion and its rate of metabolic clearance. The posthepatic metabolic clearance rate in obesity is generally normal,[7,8] whereas the efficiency of hepatic insulin extraction is unclear.[9–11] In addition, studies *in vivo* and *in vitro* have shown that insulin secretion is enhanced in obese individuals, and hypersecretion has been demonstrated in the fasting state, after glucose infusion or ingestion, and over 24 h on a mixed diet.[12–14] Secretion is in turn regulated by complex stimulatory and inhibitory influences on the β-cells. Among the inhibitory influences is the still controversial question of a direct or indirect negative feedback of circulating insulin on β-cell secretion. Insulin feedback inhibition has been reported in normal humans under euglycemic conditions,[15] although this has not been observed in other studies.[16,17]

In obesity, the insulin resistance of peripheral tissues may be paralleled by resistance to the inhibitory effects of insulin on β-cells. Graves' disease is accompanied by peripheral insulin resistance and impaired insulin feedback,[18] whereas in type 2 diabetic patients the results are divergent.[20,21] Recently, an increase in basal insulin concentrations was reported in knockout mice with lacking insulin receptor in pancreatic β-cells.[22] This finding suggests that insulin resistance in β-cells may contribute to fasting hyperinsulinemia. In obese humans some studies have reported a lack of insulin

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feedback,[17,19] whereas others have observed a level of inhibition similar to that in lean subjects following insulin infusion.[23,24] The extent of obesity and/or the magnitude of insulin resistance may be important factors in explaining these differences.

Since the morbidly obese usually show a severe peripheral insulin resistance, the present study was designed to examine the impact of experimentally induced hyperinsulinemia under euglycemic conditions on insulin secretion in this group of individuals compared to healthy, ideal weight subjects.

Material and methods

Study population

Twelve obese subjects (eight women and four men) and 16 lean control subjects (10 women and six men) were studied. None of the subjects had lost weight or changed their dietary habits during the 6 months preceding the study. The anthropometric characteristics are given in Table 1. All subjects had normal resting arterial blood pressure levels, as defined by JNC V (systolic <140 mmHg and diastolic <90 mmHg),[25] and normal glucose tolerance in an oral glucose tolerance test (OGTT) according to the National Diabetes Data Group criteria.[26] None of the subjects were on any medication which could influence insulin secretion and sensitivity. All subjects had normal liver and renal function tests. Morbid obesity was defined as a body mass index (BMI) ≥ 40 kg/m^2. The investigation was approved by the Institutional Review Board of the Faculty of Medical Sciences (UNICAMP), and all subjects gave informed consent before the study began.

Experimental protocol

Body composition was evaluated by electrical bioimpedance[27] using a Biodynamics monitor. The waist was measured at the umbilicus level and the hip at the larger buttock circumference point. Each subject received an OGTT and a euglycemic insulin clamp on different days, approximately 1 week apart. Arterial blood pressure was measured by mercury sphygmomanometry (a large cuff was used in obese individuals). For the OGTT, 40 g/m^2 of glucose was ingested over 5 min, and venous blood was sampled at 30 min intervals over 2 h for plasma glucose and insulin measurements. The clamp study, which was done after an overnight fast (12–14 h), consisted of 2 h of euglycemic insulin infusion at a rate of 280 pmol/min/m^2 of body surface area.[28] A polyethylene, 20-gage catheter was inserted into an antecubital vein for the infusion of insulin and glucose. Another catheter was inserted retrogradely into a wrist vein, and the hand placed in a heated box ($\sim 60^\circ$C) to allow the sampling of arterialized blood.[29] The 2 h before the start of the insulin infusion constituted the basal period. During the basal period and the insulin clamp, indirect calorimetry was done using a computerized, continuous open-circuit system with a canopy (Metabolic Cart Horizon, Vmax SensorMedics). Throughout the study protocol, patency of the sampling catheter was maintained by injecting 1 ml of saline after collecting each blood sample. During the insulin infusion, glucose was measured at 5 min intervals and plasma glucose was maintained with a variable glucose infusion. Venous samples for C-peptide and insulin measurements were obtained at 20 min intervals from time 20 min before until 2 h after starting the insulin infusion.

Table 1 Anthropometric characteristics of lean and obese groups

	Obese	*Lean*	P
Sex (M/F)	4/8	6/10	NS
Age (y)	38.9 ± 2.8	30.6 ± 2.8	0.02
Body weight (kg)	149.9 ± 7.8	61.1 ± 1.8	< 0.0001
BMI (kg/m^2)	54.8 ± 2.5	22.0 ± 0.5	< 0.0001
Waist/hip ratio	0.94 ± 0.05	0.78 ± 0.02	0.009
Fat-free mass (kg)	82.9 ± 3.6	49.2 ± 2.3	< 0.0001
Fat mass (%)	44.3 ± 1.5	19.0 ± 1.9	< 0.0001

BMI, body mass index; *P*, unpaired *t*-test between obese patients and lean controls.

Analytical procedures

Plasma glucose was measured by the glucose oxidase technique in a Beckman glucose analyzer (Beckman, Fullerton, CA, USA). Plasma concentrations of insulin and C-peptide were measured by radioimmunoassay using a specific kit for human insulin (less than 0.2% cross-reactivity with proinsulin) and for C-peptide (Linco Research Inc., St Louis, MO, USA). Plasma uric acid, total cholesterol, HDL cholesterol and triglycerides were assayed spectrophotometrically on an automatic colorimetric system (Cobas Miras-Roche).

Data analysis

Whole-body glucose utilization (or *M* value) was calculated from the infusion rate of exogenous glucose (GIR) during the second hour of the insulin clamp period, after correction for changes in glucose levels in a distribution volume of 250 ml/kg. The *M* value was normalized per kg of fat-free mass (μmol/min/kg FFM). An index of insulin sensitivity (IS) was calculated as the ratio of the insulin-mediated glucose clearance rate ($=M$ divided by steady-state plasma glucose level) to the steady-state plasma insulin concentration (log transformed). Areas under the OGTT time–concentration curves were calculated by the trapezoidal rule. The posthepatic insulin clearance rate was calculated as the ratio of the insulin infusion rate to the difference between the steady-state plasma insulin concentration and the product of the fasting insulin level and the ratio between steady-state C-peptide and fasting C-peptide values (insulin clearance = insulin infusion rate/(SSPI−(FPI×SSPCPep/FPCpep))).[23,30] The fasting posthepatic insulin delivery rate was obtained as the product of insulin clearance and the fasting plasma insulin concentration.[30] Post-OGTT

posthepatic insulin delivery was calculated as the product of insulin clearance and the OGTT insulin area under the curve on the assumption that insulin clearance was unchanged during glucose absorption.[31] The insulin/glucose ratio was calculated as the ratio of post-OGTT insulin delivery to post-OGTT glucose area.

Statistical analysis

All data are given as the mean ± s.e.m. Comparison of the means was done using a paired or unpaired *t*-test as appropriate. Simple linear and stepwise regression analysis and Pearson partial correlation coefficients were calculated using standard techniques. A P-value ≤ 0.05 indicated significance.

Results

Anthropometric characteristics

The patients were morbidly obese, with a BMI of $54.8 \pm 2.5\,kg/m^2$. The fat mass was very high in the obese group (about 45% of body weight) with a FFM of 82.9 ± 3.6 *vs* 49.2 ± 2.3 kg in the lean group ($P < 0.0001$). The waist-to-hip ratio was also higher in the obese group (Table 1).

Metabolic characteristics

All study subjects had a normal glucose tolerance and the area under the glucose curve during the OGTT was similar between the groups. On the other hand, fasting plasma insulin, fasting and post-OGTT insulin delivery, the area under the insulin curve, and the insulin/glucose ratio after glucose ingestion were all significantly higher in the obese than in the lean controls. Serum uric acid and plasma triglycerides were significantly higher in obese than in lean individuals (Table 2).

Clamp data

Fasting plasma glucose levels were similar in the groups, and during the clamp varied $1.5 \pm 1.2\%$ and $-1.9 \pm 0.8\%$ from the basal values in the obese and lean groups, respectively. For this reason, plasma glucose did not change significantly during insulin infusion compared to the fasting state. Fasting insulin levels were significantly higher in the obese (328 ± 94 *vs* 88 ± 4 pmol/l, $P = 0.006$) and increased to similar levels during the clamp period. IS (M value or insulin sensitivity index) was significantly impaired in the obese: the insulin-mediated glucose uptake normalized to FFM was about 40% of the lean glucose uptake (Table 3 and Figure 1). The insulin clearance was higher in obese than in lean subjects. Post-hepatic insulin delivery was also higher in the former than in

Table 2 Metabolic characteristics of the study subjects and OGTT data

	Obese	*Lean*	P
Fasting plasma glucose (mmol/l)	5.2 ± 0.2	5.1 ± 0.1	NS
Fasting plasma insulin (pmol/l)	328 ± 94	88 ± 4	0.007
Glucose area (mmol/l/2 h)	945 ± 41	849 ± 35	NS
Insulin area (mmol/l/2 h)	167 ± 29	71 ± 6	0.002
Post-OGTT insulin delivery (nmol/l/2 h)	148.1 ± 22.2	43.2 ± 4.6	< 0.0001
Insulin/glucose ratio (nmol/l mol)	158 ± 24	51 ± 5	< 0.0001
Triglyceride (mmol/l)	1.53 ± 0.13	0.71 ± 0.07	< 0.0001
Serum uric acid (μmol/l)	484 ± 46	274 ± 21	0.0004
Total cholesterol (mmol/l)	4.86 ± 0.21	4.44 ± 0.34	NS
HDL Cholesterol (mmol/l)	0.87 ± 0.05	1.23 ± 0.11	0.007
LDL cholesterol (mmol/l)	3.06 ± 0.28	2.72 ± 0.28	NS

Glucose and insulin area, area under the curves during OGTT; insulin/glucose ratio, ratio of post-OGTT insulin delivery to post-OGTT glucose area. *P*, unpaired *t*-test between obese patients and lean controls.

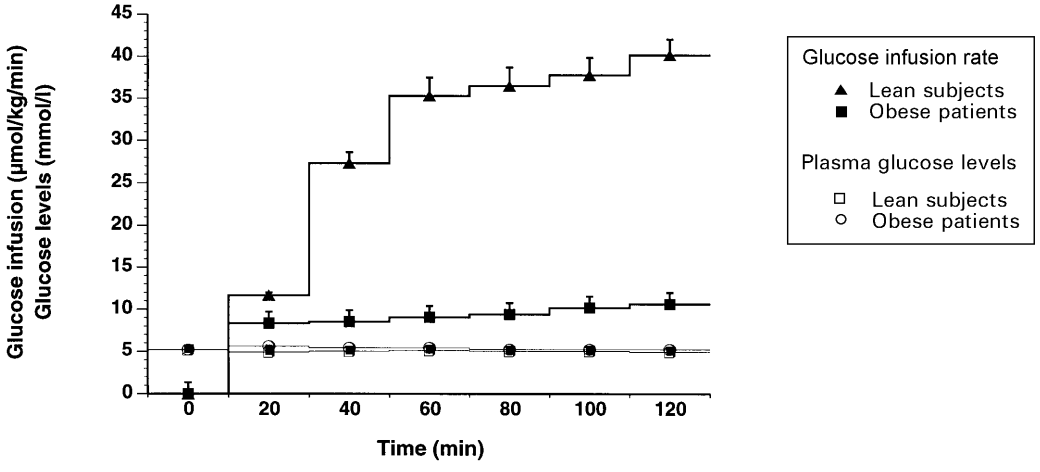

Figure 1 Glucose infusion rate during the euglycemic insulin clamp ($P < 0.0001$; ANOVA for repeated measures). Plasma glucose levels (P = NS; ANOVA for repeated measures).

Table 3 Clamp data

	Obese	*Lean*	P
Steady-state plasma glucose (mmol/l)	5.3±0.1	5.0±0.1	NS
Steady-state plasma insulin (pmol/l)	1092±190	861±54	NS
Insulin clearance (ml/min)	994±84	623±35	0.0004
Fasting insulin delivery (pmol/min)	285.6±50.8	54.7±3.3	<0.0001
M (μmol/min kg FFM)	19.8±1.6	48.7±2.6	<0.0001
IS (ml/min kg FFM/ln (pmol/l))	0.56±0.06	1.47±0.07	<0.0001
Resting energy expenditure (kcal/day)	2224±94	1451±56	<0.0001
Resting energy expenditure (kcal/FFM)	27.2±1.3	29.3±1.1	NS

M, M value from the clamp (60–120 min) normalized per kilogram of fat-free mass; IS, insulin sensitivity index; *P,* unpaired *t*-test between obese patients and lean controls.

the latter, both in the fasting state and following glucose ingestion (Tables 2 and 3).

Fasting and steady-state plasma C-peptide levels were about three times higher in the obese compared to the lean group (Table 4). The percentage decrease throughout the insulin infusion period was lower in the obese (mean 0–40 min: 10.8±10.1% *vs* −31.0±4.6%; mean 60–120 min: −16.9±6.9% *vs* −43.0±5.6%, $P<0.006$; Figure 2A, B). The fasting C-peptide-to-insulin molar ratio was similar in both groups, but decreased less during insulin infusion in the obese. As a result, during the clamp period, the molar ratio was significantly higher in these patients (Table 4).

In the lean group, the percentage variation of C-peptide was related to *M*/FFM during the clamp time from 0 to 60 min ($r=0.61$, $P=0.05$) and during the clamp steady-state period ($r=0.53$, $P=0.03$; Figure 3). These correlations remained significant even after adjustment for the glucose variation (Pearson partial correlation coefficient: 0.53, $P=0.03$). There was no correlation between C-peptide levels and age, sex or glucose levels in either group. There was also no correlation between the C-peptide levels and IS in obese subjects.

Table 4 C-peptide results during the clamp study

	Obese	*Lean*	P
Fasting plasma C-peptide (pmol/l)	1425±131	550±63	<0.0001
C-peptide 20–40 min clamp (pmol/l)	1407±135	364±46	<0.0001
Steady-state plasma C-peptide (pmol/l)	1218±204	315±47	<0.0001
Fasting C-peptide/insulin ratio	5.75±0.71	6.43±0.75	NS
Steady-state C-peptide/insulin ratio	1.16±0.10	0.39±0.06	<0.0001
C-peptide/insulin ratio (% variation)[a]	−75.9±4.1	−94.2±0.5	<0.0001

P, unpaired *t*-test between obese patients and lean controls. [a]Percentage variation of clamp C-peptide/insulin ratio to fasting ratio.

Discussion

The obese patients in this study showed many expected metabolic abnormalities, including severe peripheral insulin resistance, increased insulin secretion, a higher rate of resting energy expenditure, and higher serum triglyceride and uric acid levels and lower HDL cholesterol. As demonstrated by Bonadonna *et al*,[6] the suppression of endogenous glucose release is virtually complete at the plasma insulin levels reached in our clamp experiments, regardless of the BMI. For this reason, IS was calculated taking into account only the glucose infusion rate, normalized to the FFM. Since the plasma insulin levels at steady-state were higher in the obese group, though not significantly, the IS was also normalized to this parameter. However, the total amount of glucose

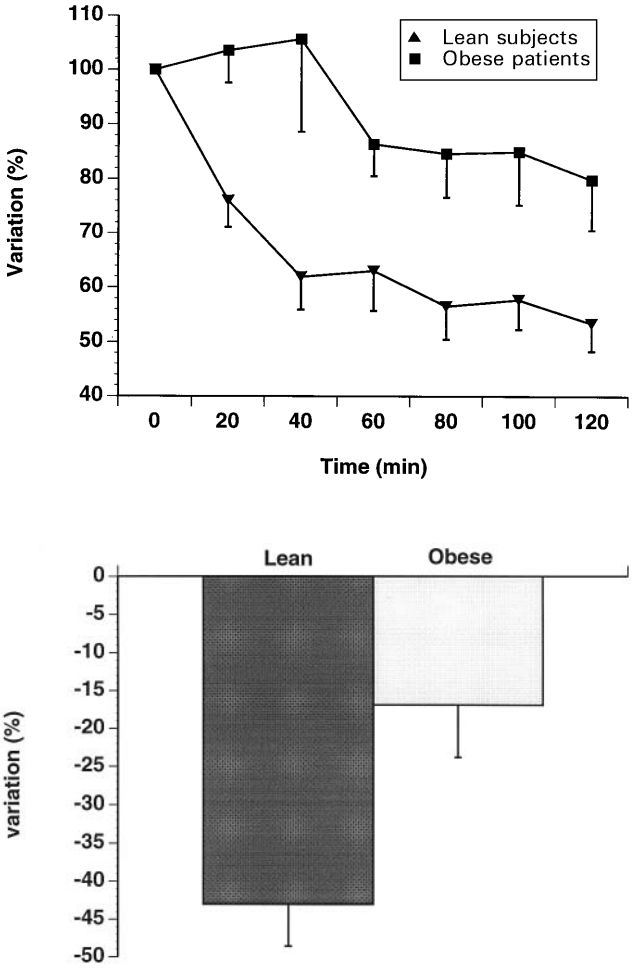

Figure 2 (A) Percentage variation in C-peptide levels during insulin infusion compared to the fasting plasma levels ($P=0.01$; ANOVA for repeated measures). (B) Percentage variation of C-peptide levels during steady-state insulin infusion (60–120 min) relative to the fasting plasma levels (obese *vs* lean; $P=0.006$, unpaired *t*-test).

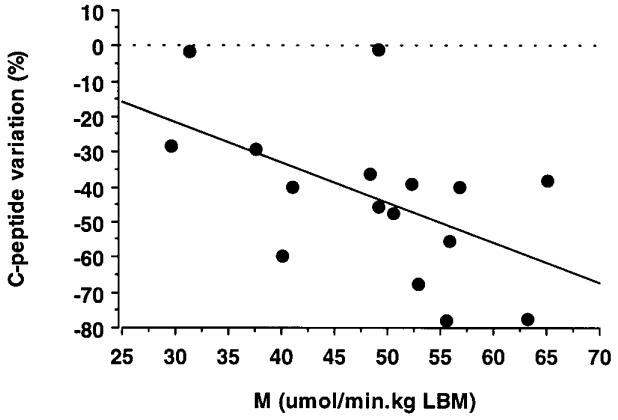

Figure 3 Relationship between the variation in C-peptide levels during steady-state insulin infusion (mean 60–120 min) and insulin sensitivity (as *M*, normalized for fat-free mass) for the lean group ($r=0.53$; $P=0.03$).

disposed of under the influence of insulin was 60% lower in obese than in lean subjects, after normalization to fat-free mass. Thus, although the increased lean mass of the obese provided a compensatory mechanism of glucose utilization, it was still very low in these patients.

Insulin secretion during a fasting and during insulin infusion in the euglycemic clamp was assessed using the C-peptide plasma levels and, during the OGTT, the posthepatic insulin delivery rate was calculated from the insulin clearance and the insulin area. C-peptide was chosen to evaluate insulin secretion because of their equivalent molar ratio secretion, the negligible hepatic extraction[32] and similar clearance in lean and obese subjects.[9] Regardless of the method used for calculation, insulin secretion was about three times higher in obese patients compared to lean subjects.

Insulin infusion under euglycemic conditions at steady-state inhibited its own secretion by about 43% in the lean. In the obese, this inhibition was blunted to about 17%. These differences were evident even in the first 40 min of the clamp. De Fronzo *et al*[33] reported a 53% reduction in insulin secretion in lean subjects with plasma insulin levels of 25 μU/ml, suggesting that the inhibition occurred at low doses of insulin infusion. No further effect was observed when the plasma insulin levels were increased. This finding suggests that the plasma insulin levels used in our study would produce a maximal response. At similar insulin infusion rates, this suppression could not be enhanced by the additional infusion of somatostatin.[34] The decrease in C-peptide (percentage variation) in the lean group was related to the IS after correction for variations in the glucose levels. The correction for glucose levels was done to minimize the effects of these variations even after arterializing the venous blood by heating the hand and attempting to maintain plasma glucose near the fasting levels. The lack of a relationship between the decrease in C-peptide levels and the plasma glucose variations is noteworthy. In the obese group there was no relationship between C-peptide variations and IS, a finding reflected in the very similar responses (*M*/LBM and C-peptide variation) to insulin infusion in these patients.

A suppressive effect of insulin on its own secretion has been seen in some studies,[15,35] but not in others.[16,17] The failure to document a fall in basal insulin secretion could be attributable to modest increases in plasma glucose that would stimulate insulin secretion, although other mechanisms may be involved. Bratusch-Marrain and Waldhäusl demonstrated that the glucose-induced insulin response in healthy subjects was unaltered by exogenous hyperinsulinemia.[35] Thus, the insulin effect on its own secretion may be more important in the fasting state. In the fasting state, the C-peptide/insulin molar ratio was similar in both groups and the amount of exogenous insulin infused was the same, so that a similar decrease was expected during the clamp. The blunted inhibition of insulin secretion could explain the elevated steady-state C-peptide/insulin molar ratio in the obese group. The plasma insulin levels reached during the clamp were slightly higher in the obese, and could be explained by a higher persistent endogenous secretion.

Posthepatic plasma insulin clearance is normal in moderately obese patients,[7,12] but was higher in the obese in our study (Table 3). Insulin clearance was not normalized to body weight or lean body mass, since insulin is not appreciably degraded by peripheral tissues,[31] and normalization can underestimate insulin clearance in the obese. When the insulin clearance was normalized to body surface area, the differences between groups were eliminated and the results obtained (data not shown) were similar to those described by Polonsky *et al*.[12] Another reason for the discrepancy with previous reports could be the type of obese subjects studied (morbidly obese, mean BMI $\approx 55\,kg/m^2$ *vs* moderately obese). Since the insulin clearance was not lower in the obese, it certainly did not contribute to the hyperinsulinemia of these patients, thus reinforcing the importance of insulin hypersecretion. Of interest was that the response of the β-cell to glucose (as expressed by the insulin/glucose ratio) was significantly enhanced in obese patients, again demonstrating hypersecretion.

The mechanism by which an increase in insulin concentration inhibits insulin secretion is not completely understood. The consistently higher endogenous C-peptide levels in the obese under both basal and suppressed conditions, and the lower percentage decrease in these subjects could have resulted from increased β-cell mass and/or insulin resistance. However, the inhibition of glucose-stimulated insulin secretion by somatostatin is maintained in the obese,[36,37] which suggests a specific defect in insulin feedback and insulin resistance. The effect of insulin on its own secretion may be indirect (neurally mediated) and/or direct, through an action of insulin on β-cells via the insulin signalling pathway. Boden *et al*[38] demonstrated that hyperinsulinemia decreased C-peptide concentrations in control subjects and in kidney transplant patients, but not in patients after combined pancreas/kidney transplantation.

The main difference between patients who received a kidney transplant and those who received a pancreas and kidney transplant was that in the latter the pancreas was denervated. These data suggest that the inhibition of pancreatic insulin secretion by hyperinsulinemia is neurally mediated. This conclusion agrees with data obtained by Stagner *et al*[39] in *in situ*-perfused, vascularly isolated but innervated canine pancreas preparations in which exogenously infused insulin produced a small suppression of C-peptide release, not seen in the completely isolated canine pancreas. Islets are innervated by sympathetic, parasympathetic and peptidergic nerves. Sympathetic and peptidergic nerves are viable candidates since their neurotransmitters, eg noradrenaline and galanin, suppress insulin secretion. In lean individuals, insulin activates the sympathetic system[40] and causes a sympathetic shift in the autonomic balance,[41] whereas in the obese, sympathetic activation during the clamp is blunted,[42,43] despite its enhanced basal activity.

Some components of the insulin signalling pathway are present in normal islet β-cells as shown by the expression of the insulin receptor and the insulin receptor substrates-1 and -2 (IRS-1, IRS-2) in rat pancreatic islets[44] and clonal β-cell lines.[45] The role of these proteins in insulin-mediated feedback of its own secretion or in insulin resistance in β-cells is unknown. However, in knockout mice lacking the insulin receptor in pancreatic β-cells, there is an increase in basal insulin concentrations at 6 months of age,[22] suggesting that insulin resistance in β-cells can contribute to fasting hyperinsulinemia. The pattern of regulation of the insulin signalling proteins in β-cells of obese patients is unknown, but in other tissues there is a widespread decrease in insulin receptor and insulin receptor substrates, and in their activation.[46] Although these proteins have a tissue-specific regulation, it is reasonable to speculate that a down-regulation of the insulin receptor and its substrates in the pancreas of morbidly obese patients may contribute to insulin resistance in β-cells of these patients.

In summary, the inhibition by insulin of its own secretion is blunted in insulin-resistant, morbidly obese patients, partially through an altered sensitivity to insulin. This alteration may have a role in the pathogenesis of fasting hyperinsulinemia in obese patients.

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PAPER

Restored insulin inhibition on insulin secretion in nondiabetic severely obese patients after weight loss induced by bariatric surgery

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OBJECTIVE: To examine the impact of important weight loss on insulin inhibition of its own secretion during experimentally induced hyperinsulinemia under euglycemic conditions.
DESIGN: Longitudinal, clinical intervention study—bariatric surgery (vertical banded gastroplasty—gastric bypass—Capella technique), re-evaluation after 4 and 14 months.
SUBJECTS: Nine obese patients class III (BMI = 54.6 ± 2.6 kg/m^2) and nine lean subjects (BMI = 22.7 ± 0.7 kg/m^2).
MEASUREMENTS: Euglycemic hyperinsulinemic clamp (insulin infusion: 40 mU/min m^2), C-peptide plasma levels, electrical bioimpedance methodology, and oral glucose tolerance test (OGTT).
RESULTS: BMI was reduced in the follow-up: 44.5 ± 2.2 and 33.9 ± 1.5 kg/m^2 at 4 and 14 months. Insulin-induced glucose uptake was markedly reduced in obese patients (19.5 ± 1.9 μmol/min kg FFM) and improved with weight loss, but in the third study, it was still lower than that observed in controls (35.9 ± 4.0 *vs* 52.9 ± 2.2 μmol/min kg FFM). Insulin-induced inhibition of its own secretion was blunted in obese patients (19.9 ± 5.7%, relative to fasting values), and completely reversed to values similar to that of lean ones in the second and third studies (−60.8 ± 4.2 and −54.0 ± 6.1%, respectively).
CONCLUSION: Weight loss in severe obesity improved insulin-induced glucose uptake, and completely normalized the insulin inhibition on its own secretion.
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Keywords: insulin resistance; insulin secretion; hyperinsulinemia; C-peptide; weight loss; bariatric surgery

Introduction

Hyperinsulinemia is an early important metabolic change in obese patients,[1–3] and has been reported as an independent cardiovascular risk factor.[4] The pancreas is able to increase its secretion precisely to compensate for the defect in insulin action to maintain normal glucose tolerance,[1,5] and hyperinsulinemia is usually considered to result from increased insulin secretion produced as a compensatory response of the B cell. However, the European Group for the Study of Insulin Resistance (EGIR) reported a more prevalent fasting posthepatic insulin delivery rate than insulin resistance in the obese subjects evaluated.[6] Insulin levels are a result of the rate of insulin secretion and of its metabolic clearance rate. In obese individuals, the posthepatic metabolic clearance rate is thought to be normal[7] and insulin secretion is enhanced.[3,8]

Insulin secretion is, in turn, determined by complex stimulatory and inhibitory influences on the β cell. Among the inhibitory influences is a direct or indirect negative feedback of circulating insulin on β-cell secretion, whose role is still controversial. In human obesity, some studies have reported a failure of insulin feedback and others have observed an inhibition similar to that in lean subjects.[9–12]

The obesity degree and/or the magnitude of insulin resistance may be important factors to justify these differences. In a previous study, we observed a failure of insulin

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feedback on insulin secretion in extreme obesity (BMI > 40 kg/m^2), while in lean subjects the insulin inhibition was directly related to insulin sensitivity.[7] Since severe obesity (obese class III[13]) usually displays serious metabolic alterations[13,14] that can be changed by weight loss,[14–17] and the effect of weight loss on the insulin inhibition on insulin secretion was not yet investigated, the present study was designed to examine the impact of experimentally induced hyperinsulinemia under euglycemic conditions on its own secretion in these patients, before and after surgically (vertical banded gastroplasty—gastric bypass—Capella technique) induced weight loss.[18,19] C-peptide, which is the connecting peptide of proinsulin, is secreted from β cells in equimolar amounts with insulin, and its concentrations have been used to determine insulin production *in vivo*. In this study, insulin secretion was evaluated by C-peptide plasma concentrations.

Material and methods

Study population

Nine obese patients (seven women and two men), aged 39 $\pm$ 4 y, were studied three times: Study I (SI)—before bariatric surgery; Study II (SII)—after weight loss of 15–20% ($\sim$4 months after surgery); Study III (SIII)—after weight stabilization (38% weight loss relative to the initial body weight; $\sim$14 months after surgery). Nine lean subjects (seven women and two male) aged 34.4 $\pm$ 4 y were used as a control group (C). The anthropometric characteristics are given in Table 1. All volunteers had normal resting arterial blood pressure levels, as defined by JNC V[20] (systolic < 140 mmHg and diastolic < 90 mmHg), and normal fasting glucose according to the American Diabetes Association criteria.[21] None of them were on any medication, which could influence insulin secretion and sensitivity. All subjects had normal liver and renal function tests and no cardiovascular or respiratory disease that could contraindicate the surgery. Class III obesity was defined as body mass index (BMI) $\geq$ 40 kg/m^2.[13] After the initial investigation, the obese patients were submitted to a vertical banded gastroplasty—gastric bypass—Capella technique.[19] The Institutional Review Board of the School of Medicine (Campinas State University) approved the investigation, and all subjects gave informed consent before the protocol began.

Experimental protocol

Body composition was evaluated by electrical bioimpedance[22] using a Biodynamic monitor. Each subject underwent an OGTT and a euglycemic hyperinsulinemic clamp on different days, approximately 1 week apart. Arterial blood pressure was measured by a mercury sphygmomanometer (a large cuff was used). For the OGTT, 40 g/m^2 of glucose was ingested over 5 min, and venous blood was sampled at 30 min intervals over 2 h for plasma glucose and insulin measurements. The American Diabetes Association criteria[21] were used to define glucose tolerance during OGTT. The clamp study, which was done after an overnight fast (12–14 h), consisted of 2 h of euglycemic insulin infusion at a rate of 40 mU/min m^2 of body surface area.[23] A polyethylene, 20-gauge catheter was inserted into an antecubital vein for the infusion of insulin and glucose. Another catheter was inserted retrogradely into a wrist vein, and the hand placed in a heated box ($\sim$60°C) to allow sampling of arterialized blood.[24] The 2-h period before the insulin infusion was started constituted the basal period. During insulin infusion glucose was measured at 5 min intervals and plasma glucose was maintained nearly constant with a variable glucose infusion. Arterialized venous samples for C-peptide and insulin measurements were obtained at 20 min intervals, from 20-min before until 2 h after starting the insulin infusion.

After an initial set of clinical and metabolic investigations, patients were submitted to a vertical banded gastroplasty—gastric bypass—Capella technique.[19] The nutritional guidelines after surgery consisted of a liquid hypocaloric diet (around 60% carbohydrate, 20% lipid and 20% protein),[18] with gradual reintroduction of solid foods and caloric increase, according to patient acceptance and postoperative period. Vitamin supplementation was started 1 month later.

The second and third metabolic studies carried out only in obese patients were similar to the first, except for OGTT, which was impossible because of gastric restriction and impaired digestive absorption.

Table 1 Anthropometric characteristics in the studies

	Control	*Study I*	*Study II*	*Study III*	P1
Body weight (kg)	60 $\pm$ 3	145 $\pm$ 9***	118 $\pm$ 7***	90 $\pm$ 5***	0.0003
BMI (kg/m^2)	22.7 $\pm$ 0.7	54.6 $\pm$ 2.6***	44.5 $\pm$ 2.2***	33.9 $\pm$ 1.5***	0.0003
Waist (cm)	75 $\pm$ 3	131 $\pm$ 6***	111 $\pm$ 7**	111 $\pm$ 6**	0.007
Free fat mass (kg)	46.8 $\pm$ 3.3	78.7 $\pm$ 3.7***	68.4 $\pm$ 3.5***	61.1 $\pm$ 2.3**	0.0008
Fat mass (%)	22.1 $\pm$ 2.5	45.4 $\pm$ 1.5***	41.8 $\pm$ 1.5***	31.8 $\pm$ 1.5*	0.0003

BMI body mass index; *P*1, *P*-value—Friedman analyses for the three studies in obese patients.
*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$—Studies I, II or III *vs* control group—Mann-Whitney analyses.

Analytical procedures

Plasma glucose was measured by the glucose oxidase technique in a Beckman glucose analyzer during the clamp study (Beckman, Fullerton, CA, USA). Plasma concentrations of insulin and C-peptide were measured by radioimmunoassay using a specific kit for human insulin (less than 0.2% cross reactivity with proinsulin) and for C-peptide (Linco Research Inc., St Louis, MO, USA). Plasma uric acid, total cholesterol, HDL cholesterol and triglycerides were assayed spectrophotometrically on an automated colorimetric system (Cobas Miras, Roche).

Data analysis

Whole-body glucose utilization (or M value) was calculated from the infusion rate of exogenous glucose (GIR) during the second hour of the insulin clamp period, after correction for changes in glucose levels in a distribution volume of 250 ml/kg. The M value was normalized per kilogram of fat-free mass (μmol/min kg Free fat mass (FFM)) and divided by the prevailing steady-state insulin plasma levels (log transformed). Areas under OGTT time–concentration curves were calculated by the trapezoidal rule.

Statistical analysis

All data are given as the mean ± s.e.m. Comparison of the means in the three studies from obese patients was done using the nonparametric Friedman analysis, and ANOVA for repeated measures was carried out to compare variables curves of obese and control groups. Comparison of means between obese and control group was done using the nonparametric Mann–Whitney *U*-test. Simple linear and stepwise regression analyses were calculated, using the *StatView* computerized program. A P-value ≤ 0.05 indicated significance.

Results

Anthropometric characteristics

The patients were extremely obese, with a BMI of $54.6 \pm 2.6\,kg/m^2$, with very high fat mass (about 45% of body weight). The weight loss induced by the surgery was very important, reaching $-18.4 \pm 0.5\%$ in SII and $-37.6 \pm 0.5\%$ in SIII. So, the anthropometric measures were quite improved together with a reduction in the body fat percentage, but were still significantly higher than control values (Table 1).

Metabolic characteristics

Seven of the obese patients had a normal glucose tolerance[21] and two had impaired glucose tolerance and normal fasting plasma glucose. The area under the glucose curve during the OGTT was similar to control (920 ± 46 *vs* 814 ± 37 mmol/l min). Fasting plasma insulin and the area under the insulin curve were significantly higher in the patients. The weight loss induced significant decreases in the fasting plasma insulin and glucose levels (Table 2). Decreases in serum uric acid (494 ± 42 *vs* 327 ± 30 μmol/l, $P = 0.009$) and in plasma triglycerides (1.67 ± 0.09 *vs* 1.10 ± 0.07 mmol/l, $P = 0.002$) were observed, while there was no significant change in the fasting total cholesterol (from 4.60 ± 0.25 to 4.26 ± 0.15 mmol/l) and HDL cholesterol (from 0.81 ± 0.05 to 1.00 ± 0.08 mmol/l).

Clamp data

The plasma glucose levels during insulin infusion varied -2.9 ± 0.4; 3.6 ± 1.0; 0.5 ± 1.2 and $-0.7 \pm 1.3\%$ from the basal values in the control and in the studies I, II and III, respectively. Thus, plasma glucose did not change significantly during the clamp period compared to the fasting state in each group study. Although the insulin infusion was the same in the studies, the steady-state plasma insulin levels were similar in the control and the obese and decreased after weight reduction ($P = 0.01$). Insulin sensitivity (M value or M/LBM) was significantly lower in the obese group and improved from 19.5 ± 1.9 to 35.9 ± 4.0 μmol/min kg FFM, $P = 0.0003$ 14 months after the surgery (Table 2).

Fasting and steady-state plasma C-peptide levels were higher in the obese before surgery than in the control and decreased after weight reduction (Table 3 and Figure 1). The percentage decrease throughout all the insulin infusion period was significantly lower in study I compared to the

Table 2 Clamp data

	Control	*Study I*	*Study II*	*Study III*	*P1*
Fasting plasma glucose (mmol/l)	5.2 ± 0.1	4.9 ± 0.1	4.6 ± 0.1**	4.6 ± 0.1**	0.02
Steady-state plasma glucose (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	4.7 ± 0.1*	4.6 ± 0.1*	0.002
Fasting plasma insulin (pmol/l)	86 ± 5	231 ± 18***	109 ± 9*	58 ± 5**	0.0009
Steady-state plasma insulin (pmol/l)	852 ± 71	889 ± 73	841 ± 57	657 ± 35*	0.01
M/FFM (μmol/min kg FFM)	52.9 ± 2.2	19.5 ± 1.9***	24.9 ± 2.2***	35.9 ± 4.0**	0.0003
M/FFM/I (μmol/min kg FFM/ins ln)	7.7 ± 0.4	2.8 ± 0.3***	3.4 ± 0.2***	5.4 ± 0.5***	0.0001

Steady-state period, from 60 to 120 min of insulin infusion; M/FFM (μmol/min kg/FFM/pmol/l), M value from the clamp normalized by kilogram of fat-free mass; M/FFM/I, M value from the clamp normalized by kilogram of fat-free mass divided by the natural logarithm of steady-state plasma insulin (pmol/l).
Pl, *P*-value—Friedman analyses for the three studies in obese patients;
*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$—Studies I, II or III *vs* control group—Mann–Whitney analyses.

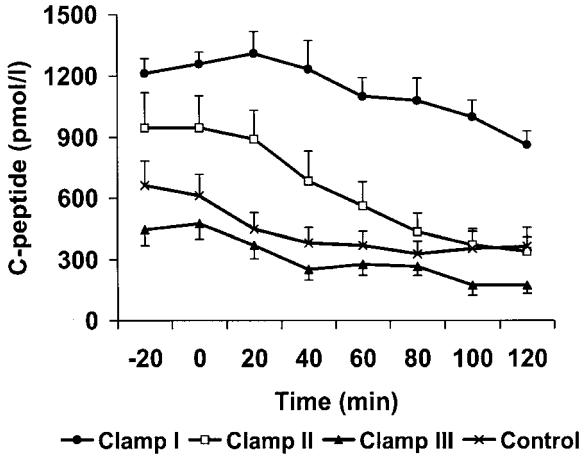

Figure 1 Plasma C-peptide levels during the euglycemic insulin clamp. $P \leq 0.0001$; ANOVA for repeated measures.

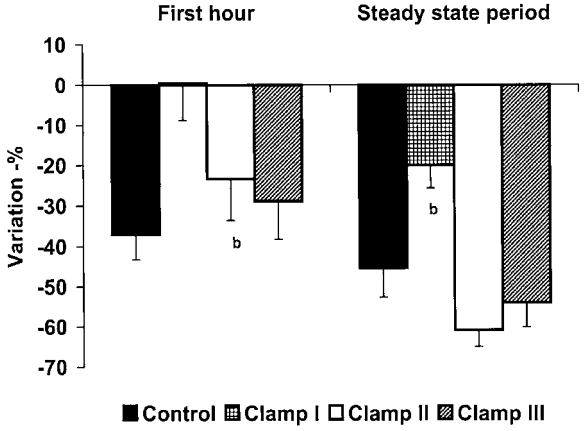

Figure 2 Percentage variation of plasma C-peptide levels during insulin infusion (first hour and steady-state periods) relative to fasting plasma levels. (b) $P \leq 0.01$ *vs* control group; $P = 0.0008$—Friedman analyses for the percent variations of steady-state period values in the three studies of obese patients.

control group and to studies II and III (Figure 2). The fasting C-peptide-to-insulin molar ratio was similar in all studies (Table 3).

In the pooled data from the three studies of the obese group, fasting plasma C-peptide levels were related to BMI ($r = 0.59$, $P = 0.001$), % fat mass ($r = 0.58$, $P = 0.002$), M/FFM ($r = -0.73$, $P < 0.0001$) and waist ($r = 0.53$, $P = 0.01$), but not to waist/hip ratio and to fasting plasma glucose ($P =$ NS). In a stepwise model (including BMI, age, M/FFM, % fat mass, sex and waist), M/FFM and percentage of fat mass remained significantly related to fasting plasma C-peptide (multiple $r = 0.82$, $P < 0.0001$; $r^2 = 0.63$).

Discussion

The obese subjects of this study had class III obesity[13] with very high BMI, fat mass and fasting plasma insulin levels and were markedly insulin resistant. The bariatric surgery induced marked weight reduction, but BMI after weight stabilization was around 34 kg/m^2. The weight reduction was accompanied by improvement in body composition, fat/free fat mass, because of a proportionally higher fat than FFM loss. The important metabolic abnormalities, such as severe peripheral insulin resistance, increased insulin secretion, high serum triglycerides and uric acid levels, all improved after weight loss.

Insulin secretion during the fasting state and under insulin infusion during the euglycemic clamp was assessed using the C-peptide plasma measurements. C-peptide was chosen to evaluate insulin secretion because of their equivalent molar ratio secretion, negligible hepatic extraction[25] and similar clearance in lean and obese subjects.[8] Regardless of the method used for calculation, insulin secretion was much higher in the obese in the first study, and the decrease after weight loss was important and statistically significant even in the second study, with further reduction in the third study. Fasting C-peptide levels were mainly related to insulin sensitivity and percentage of fat mass, as demonstrated by the stepwise model including BMI and M value. These results again demonstrate the well-known role of insulin resistance and obesity to determine fasting insulin secretion.

Table 3 C-peptide results during the clamp studies

	Control	*Study I*	*Study II*	*Study III*	P*1*
Fasting plasma C-peptide (pmol/l)	634±106	1235±60**	946±164*	471±85	0.008
Plasma C-peptide 0–60 min (pmol/l)	401±71	1227±105***	713±129*	313±52	0.0008
Steady-state plasma C-peptide (pmol/l)	350±77	980±67***	384±79	207±43	0.001
Clamp variation 0–60 min (%)[a]	−37.0±6.3	0.4±9.2**	−23.3±10.3	−28.8±9.4	ns
Clamp variation 60–120 min (%)[a]	−45.3±7.4	−19.9±5.7**	−60.8±4.2	−54.0±6.1	0.0008
Fasting C-peptide/insulin ratio	7.5±1.2	5.5±0.4	8.0±1.1	8.6±1.6	ns

Plasma C-peptide 0–60 min—mean C-peptide values from plasma samples collected at 20, 40 and 60 min of insulin infusion; Steady-state plasma C-peptide—mean plasma C-peptide values from plasma samples collected at 80, 100 and 120 min of insulin infusion;
[a]Percent variation of clamp to fasting values.
*P*l, *P*-value—Friedman analyses for the three studies in obese patients.
*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$—Studies I, II or III *vs* control group—Mann-Whitney analyses.

Some of the recently described factors secreted by the adipocytes have actions on insulin secretion and/or insulin sensitivity, such as leptin, adiponectin, TNFα. Some authors suggest that leptin can inhibit insulin secretion[27] and its plasma levels markedly decrease with weight reduction. It is not possible to know if plasma leptin had some influence on the insulin secretion in the present study. In our surgery department, other obese patients submitted to the same bariatric surgery had reduced leptin and insulin plasma levels, but only BMI was related to the latter in a multivariate regression analysis.[28] In addition, some studies have reported that the interaction between leptin and insulin depends on factors such as leptin concentration, exposure time and glucose concentration. Thus, the functional role played by leptin on insulin secretion is still under discussion.

A suppressive effect of insulin on its own secretion has been seen in some studies[26,29] but not in others.[30,31] In a previous study, insulin infusion under euglycemic conditions at steady state inhibited its own secretion in lean subjects but this insulin action was blunted in the obese group.[7] In this study we observed that in severely obese patients insulin-induced inhibition of insulin secretion was blunted before surgery (SI) compared to the control group, suggesting insulin resistance to this effect. After weight loss, the decrease in C-peptide during insulin infusion improved. The percentage variation of C-peptide levels during steady state or in the first hour of the clamp, relative to fasting plasma levels, 14 months after the surgery were similar to those observed in lean subjects. It is interesting to note that 4 months after surgery or only after 15–20% reduction in BMI, although the basal C-peptide levels were still higher than controls, the percentage variation of C-peptide levels during steady-state insulin infusion relative to the fasting plasma levels was similar to control levels, showing an early recovery.

The results of the present study allowed us to compare two different effects of insulin in severely obese patients and after weight loss. Insulin-induced glucose uptake, which was reduced in these patients, improved after weight loss, but was still lower than that observed in controls. The lower steady-state insulin plasma levels, in Study III, can be explained by the decreased endogenous insulin secretion after weight loss as suggested by the C-peptide evaluation. Thus, the improvement of the insulin sensitivity could be underestimated but the correction of M value by the prevailing insulin plasma levels demonstrated a still lower sensitivity in the obese after weight reduction. On the other hand, insulin-induced inhibition of its own secretion was completely reversed to values very similar to those observed in lean controls after weight loss. These results demonstrate for the first time that weight loss restores the insulin action on its own secretion and suggest that it has an earlier recovery after weight loss than glucose utilization, or that the first effect is only disturbed in situations of severe insulin resistance.

In obesity, the mechanism of insulin resistance, insulin hypersecretion and impaired insulin inhibition on its own secretion are not completely understood. The inhibition of glucose-stimulated insulin secretion by somatostatin is maintained,[32] which suggests a specific defect in insulin feedback, even if there is evidence that functioning β cell mass is enhanced.[33] The effect of insulin on its own secretion may be indirect (neurally mediated) and/or direct, through an action of insulin on β cells via the insulin-signaling pathway. Previous data suggest that the inhibition of pancreatic insulin secretion by hyperinsulinemia is neurally mediated.[34] In lean individuals, insulin activates the sympathetic system[35] and causes a sympathetic shift in the autonomic balance, whereas in the obese sympathetic activation during the clamp is blunted,[36] despite its enhanced basal activity.[37] Accordingly, after BMI reduction in the obese there is also evidence of improvement in the sympathetic activation by insulin.[38]

Some components of the insulin-signaling pathway are present in normal islet β cells.[39,40] And, in knockout mice lacking the insulin receptor in pancreatic β cells there is an increase in basal insulin concentrations,[41] suggesting that insulin resistance in β cells can contribute to fasting hyperinsulinemia. The pattern of regulation of the insulin signaling proteins in β cells of obese patients is unknown, but in other tissues there is a widespread decrease in insulin receptor and insulin receptor substrates, and in their activation. Although these proteins have a tissue-specific regulation, it is reasonable to speculate that a downregulation of the insulin receptor and its substrates in the pancreas of severely obese patients may contribute to insulin resistance in β cells of these patients, and that weight loss may control these alterations.

We concluded that insulin inhibition on its own secretion is blunted in insulin-resistant severely obese patients, partially related to the degree of obesity, and weight loss completely normalizes the insulin effect on its own secretion.

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Title: Insulin secretion and insulin resistance in non-diabetic morbidly obese patients: effect of weight loss induced by bariatric surgery

Short running title: Enhanced insulin action after bariatric surgery

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Abstract

Objective: to evaluate insulin action on metabolic pathways and insulin secretion in non-diabetic class III obese after weight loss by bariatric surgery.

Research methods and procedures: 13 obese patients (4M/9F; BMI=56.3±2.7 $kg.m^{-2}$) and 13 lean subjects (5M/8F; BMI=22.4±0.5 $kg.m^{-2}$) were submitted to euglycemic clamp, oral glucose tolerance test (OGTT) and indirect calorimetry. The study was carried out before (*study I*) and after weight loss (about 40% relative to initial body weight – *study II*) induced by vertical banded gastroplasty - gastric bypass.

Results: The obese were insulin resistant (M=19.0±1.4 vs. 51.5±2.4 umol/min.kgFFM, p<0.0001) and hyperinsulinemic at fasting state (332±86 vs. 85±5 pmol/l, p<0.0001) and during the OGTT compared to the lean subjects. Insulin secretion normalized after weight loss, while M increased (35.5±3.7 umol/min.kgFFM, p<0.05 vs. study I). The higher insulin clearance of the obese did not change in the follow-up. Insulin induced glucose oxidation and non-oxidative glucose disposal, NOGD, were lower in the obese compared to the lean (al p<0.05). In study II, the former increased slightly while the NOGD reached values similar to those of the lean group. Fasting lipid oxidation was higher in the obese than in the lean, and did not change significantly in study II. Lipid oxidation under insulin effect was a little increased after weight loss (p=0.01) and inversely related to glucose oxidation (r=-0.78; p-0.0003).

Discussion: the rapid weight loss in obese class III, after surgery, induced a normalization of insulin secretion and only an improvement of insulin sensitivity, almost entirely dependent of glucose storage.

Keywords: insulin resistance, insulin secretion, insulin clamp, glucose metabolism, obesity and bariatric surgery.

Introduction

Obesity, especially class III (1) or morbid obesity (BMI > 40 kg/m^2, about 5% of obese individuals) has been recognized as etiological or as a contributing factor to several adverse health problems such as coronary heart disease (2), dyslipidemia (3,4), sleep apnea (5), insulin resistance (6), hyperinsulinemia (7) and type 2 diabetes (8,9).

A new goal of obesity management has postulated that a weight loss of 5% to 10% of the initial body weight is frequently sufficient to improve weight-related complications (10), but it is not clear which percentage is sufficient for the morbidly obese (11). Many of these complications have been associated to insulin resistance. The link between excess weight and insulin resistance has been recognized for many years (12), but the precise etiology is not totally known. A large number of studies have shown important reductions in the incidence of diabetes (13), hyperinsulinemia and insulin resistance associated to clinically induced weight loss (14,15).

Metabolic (16) and cardiovascular improvement and reduction of most of the co-morbidities (17) have been repeatedly described after bariatric surgery. Some studies have demonstrated a normalization of insulin sensitivity in parallel to the achievement of ideal body weight (18,19). A full reversion of insulin resistance, despite BMI still being in the obese range has been reported (20,21), but Burstein et al did not find insulin sensitivity normalization, in spite of significant weight reduction (22). These controversial results suggest that the chosen strategy to achieve weight reduction, in addition to the amount of weight lost, is important to metabolic improvement.

Among the surgical techniques, there are specific differences relative to the remaining volume of the proximal stomach and partial selective induced malabsorption (23). A combination of restrictive and malabsorptive procedures, vertical banded gastroplasty – gastric bypass (Roux Y) has been frequently used (24). It would be interesting to follow the metabolic changes after weight reduction induced by this technique, since is not clear which of the insulin related pathways can be restored by the rapid weight loss.

The aim of this study was to evaluate insulin sensitivity, insulin secretion and substrate utilization before and after weight loss in obese patients (class III) submitted to the above surgery. A control group of normal weight volunteers, paired according to age and gender, has also been evaluated.

Material and Methods

Study population – Morbidly obese subjects (OB- 9 women and 4 men; aged 38.5 ± 2.6 y) and 13 lean control subjects (CT- 8 women and 5 men aged 33.0 ± 2.0 y) were evaluated. None of the subjects had lost weight or changed dietary habits during the 6 months preceding the study. The anthropometric characteristics are given in table I. All subjects had normal resting arterial blood pressure levels according to the JNC V (25) (systolic < 140 and diastolic < 90 mm Hg) and normal fasting glucose according to the revised ADA criteria (26). None were taking any medication, which could influence insulin secretion and sensitivity. All subjects had normal results in liver and renal function tests. Class III obesity was defined by Body Mass Index, BMI, ≥ 40 $Kg.m^{-2}$. The Institutional Review Board of the Medical School - UNICAMP approved the investigation, and all subjects gave informed consent before the study began.

After the initial metabolic studies (*study I*) the patients were submitted to a vertical banded gastroplasty - gastric bypass. The clamp study and the anthropometric measurements were repeated in eleven of them after a weight stabilization was achieved (~ 14 months; weight loss of 38 ± 1%; *study II*).

Experimental protocol – metabolic studies – In all study subjects, body composition was evaluated by electrical bioimpedance using a Biodynamics monitor, and the same physician measured the waist and hip circumferences. An oral glucose tolerance test and an euglycemic insulin clamp were carried out approximately 1 week apart. Arterial blood pressure was measured by mercury sphygmomanometer (in obese individuals a large cuff was used). For the OGTT, 40 g/m^{-2} of glucose was ingested, and venous blood was sampled at 30-min intervals for 2 h for plasma glucose and insulin measurements. The

clamp study, which was carried out after an overnight (12-24 h) fast, consisted of 2 hours of euglycemic insulin infusion at a rate of 280 pmol.min-1 per m^2 of body surface area (27), and was preceded by a 2h control period. A polyethylene, 20-gauge catheter was inserted into an antecubital vein for the infusion of insulin and glucose. Another catheter was retrogradely inserted into a wrist vein and the hand placed in a heated box (~60°C) for the sampling of arterialized blood (28). During the basal period and the insulin clamp, an indirect calorimetry was performed using a computerized, continuous open-circuit system with a canopy (Metabolic Cart Horizon, Vmax SensorMedics). During the insulin infusion, glucose was measured at 5 min intervals and plasma glucose was maintained by means of a variable glucose infusion, using an infusion pump (AVI 370-3M). Venous blood was sampled at 20-min intervals from time –20min until 2 hours after insulin infusion was started for C-peptide and insulin measurements.

Analytical procedures – Plasma concentrations of insulin and C-peptide were measured by radioimmunoassay using a specific kit for human insulin, with less than 0.2% of cross reactivity with pro-insulin, and for C-peptide (Linco Research Inc., St Louis, USA). Plasma glucose was measured by the glucose oxidase technique in a Beckman Glucose Analyzer (Beckman, Fullerton, CA). Plasma uric acid, total cholesterol, HDL cholesterol and triglycerides were assayed in duplicate, spectrophotometrically, on a colorimetric automatic system (Cobas Miras – Roche). Urinary non-protein nitrogen concentrations were measured by the Kijeldhal method.

Data Analysis - Whole-body glucose utilization (or M value) was calculated from the infusion rate of exogenous glucose (GIR) during the 2nd hour of the insulin clamp period, after correction for changes in glucose levels in a distribution volume of 250 $ml.kg^{-1}$. The M value was normalized per kg of fat-free mass ($\mu mol.min\text{-}1.kg\ FFM^{-1}$) and per the insulin plasma level at steady state period M/FFM.I.. Areas under OGTT time-concentration curves were calculated by the trapezium rule. The post-hepatic insulin clearance rate was calculated as the ratio of insulin infusion rate to the difference between the steady-state plasma insulin concentration and the product of fasting insulin level and the ratio between steady state C-peptide and fasting C-peptide values (Ins Clear. = Ins. Inf. Rate/(SSPI - (FPI.SSPCPep/FPCpep)))(29). Substrate utilization was calculated from the

carbon dioxide release, oxygen consumption and nitrogen urinary excretion, as previously described. Non-oxidative glucose disposal was calculated as the difference between whole body glucose uptake and net glucose oxidation (30).

Statistical Analysis – All data are given as mean ± SEM. Mean comparison between lean and obese groups was performed by Mann-Whitney U test, whereas paired comparison were performed using Wilcoxon signed rank test. ANOVA for repeated measures, Spearman and correlation stepwise regression analysis were carried out by standard techniques. A p value ≤ 0.05 was considered to be statistically significant.

Results

Anthropometric characteristics – The patients were morbidly obese subjects with body mass index of 56.3 ± 2.7 $kg.m^{-2}$. The fat mass was very high, about 45 % of body weight, and fat free mass was 84 ± 4 *vs*. 48 ± 3 kg in the lean group, $p<0.0001$. The waist circumference was also greater in the obese group. The weight reduction was very important but even after weight stabilization the patients were still obese, BMI = 34.7 ± 2.1 kg/m^2.

Metabolic characteristics – All study subjects had normal glucose tolerance and the area under the glucose curve during the OGTT was similar between the OB and CT groups (938 ± 38 *vs*. 820 ± 48 mmol/l). On the other hand, fasting plasma insulin and the area under the insulin curve were significantly higher in the obese before surgery than in CT (152 ± 23 *vs*. 65 ± 6 pmol/l). Fasting insulin and glucose were significantly lower after weight reduction.

Serum uric acid and plasma triglycerides were significantly higher in the obese patients, whereas plasma HDL cholesterol was lower. In addition, serum uric acid and triglycerides decreased in study II and HDL cholesterol increased, although not significantly, and was still lower than CT group (Table 1).

Clamp data – The whole body glucose uptake, M value, was significantly impaired in the obese (study I: 19.7 ± 1.5 *vs*. lean group: 51.5 ± 2.4 umol/min.kgFFM; p< 0.0001) and increased significantly after weight loss (35.5 ± 3.7 umol/min.kgFFM; p< 0.05 *vs*. CT and study I) (Fig 1).

The lower fasting glucose oxidation rate in the obese group compared to the lean was not statistically significant, but became so after weight loss. Insulin stimulated glucose oxidation was higher in the lean group compared to the obese (p = 0.0009) and this response improved slightly after weight loss (study II *vs*. study I – p = 0.03). The non oxidative glucose disposal, during clamp steady state, was reduced in the obese group compared to the lean (less than 50%), improving significantly after weight loss to similar levels as those found in the lean group (Table 2).

Fasting protein oxidation was reduced and did not change after weight loss. Suppression of protein oxidation by insulin infusion was similar between lean and obese and was not influenced by weight reduction (Table2)

As can be seen in table 2, the basal rate of lipid oxidation did not change with weight reduction and was still higher in the obese group than in the lean (both p<0.05). Suppression by insulin did not reach statistical significance in the lean subjects. In obese study I, lipid oxidation was slightly stimulated by insulin (p=0.06 vs. lean), but in study II, insulin infusion induced a slight suppression, similar to that of the control group (p=0.03 *vs*. Study I). In addition, an inverse relationship was observed between the insulin-induced changes in lipid oxidation and glucose oxidation (obese studies II and I: r = -0.78; p = 0.0003).

Fasting respiratory quotient, QR, was lower in the obese compared to the lean group and decreased even more after weight loss (all p<0.05). Insulin infusion induced a QR increase in lean and obese subjects - study II, but not before surgery. Therefore, the insulin effect was different in study I relative to the lean and study II (p = 0.001).

Energy expenditure - resting energy expenditure, REE, was higher in the obese patients, but not when normalized by FFM. The resting energy expenditure decreased significantly after weight loss, and was still higher than that of the lean subjects. However, the resting energy expenditure was normalized by body weight, but not by FFM, increased significantly in study II (table 3). The insulin-induced thermogenesis was similar in lean and obese subjects and did not change after weight reduction, as demonstrated in Table 3.

Discussion

The patients enrolled in this study were morbidly obese and by selection criteria had normal glucose tolerance. As expected, compared to the lean, they had higher triglyceride and plasma uric acid and lower HDL cholesterol. In addition, a severe insulin resistance was found associated to a very high insulin secretion. The whole body insulin disposal, under the insulin infusion, was very low in these patients, about 40% of the CT, and was present in lipid and in glucose metabolic pathways (oxidative and non oxidative disposal). It is important to note that weight loss was associated to a complete normalization of insulin secretion but only to an improvement in insulin resistance. Furthermore, the non-oxidative glucose disposal (NOGD) was the only metabolic pathway that improved to levels comparable to those of lean subjects, while glucose oxidative utilization improved slightly and fasting lipid oxidation remained higher than those of the lean.

Obese patients submitted to bariatric surgery were chosen for the study because this procedure is highly efficient to induce weight loss. It is important to note that after a 40% weight loss relative to their initial body weight, and one year later, the patients were still obese (BMI ~ 35kg.m^{-2}). In this way, the still low insulin sensitivity compared to the control group could be expected. Furthermore, the results relative to insulin resistance match those previously described in this body mass index range. In the patients studied by Mingrone *et cols*. (18), insulin sensitivity two years after biliopancreatic diversion, which induces a predominantly lipid malabsorption, was similar to that of the lean. A possible explanation can be related to different lipid absorption rates following the surgical

techniques used. It can be observed that final BMI was normal in that study, while not in ours. Our results are in agreement with Burstein *and cols.* (22) evaluating a small mixed group (diabetics and normoglycemics).

The fasting insulin secretion and that stimulated by glucose ingestion were three times higher in obese compared to lean subjects. Despite persistent insulin resistance, after weight reduction, normalization in the insulin secretion was achieved. Thus, the insulin secretion and the insulin sensitivity improvements had different degrees and time courses. These data suggests that insulin hypersecretion was not only compensation for insulin resistance, as suggested before. Other mechanisms should be associated. Insulin clearance seems not to contribute to the hyperinsulinemia since in these obese subjects it was higher than in the lean. The present study does not explain the mechanisms by which the insulin hypersecretion and hyperinsulinemia normalize while the insulin sensitivity improves but does not normalize. It is possible to speculate that some gut mechanism or low food ingestion and/or absorption can be implicated. Greco *et cols*. (31) suggested that the low fat absorption and circulating lipids were linked to changes in intramyocellular fat depots in some way and to the normalization of insulin action in their patients. If this is a consequence of the surgery technique used, it is not clear. The hormonal and cytokines secreted by the adipose tissue can have an important role in the consequences of weight reduction. Once the weight loss of these patients stopped, it was not possible to know if the insulin sensibility could normalize if their BMI decreased to 25kg.m^{-2} or even less.

Impaired glucose storage represents the main determinant of insulin resistance in obese subjects with normal glucose tolerance (32). We observed a very low non-oxidative glucose disposal before surgery and a total recovery after weight loss. It is noteworthy to observe the very low response of the glucose oxidative pathway to weigh loss in a clear contrast to the non-oxidative glucose utilization in our patients. These findings are in agreement with those reported after a very low- calorie diet for 6 weeks, in Class I obese patients (33). The mechanisms triggered by weight loss that impact more in non-oxidative glucose disposal than in oxidative are not clear. An interesting result is the inverse relationship between the insulin actions in glucose lipid oxidation, in the pooled data of obese studies II and I.

In fact, before surgery the lipid oxidation was higher in the obese and did not display any change under insulin administration, in opposition to the lean response. These results could suggest a persistent resistance to the insulin effect on lipid oxidation after weight loss. A previous study accessing intermediary metabolites during insulin infusion has suggested that weight reduction, in an early follow-up, improved the sensitivity of glucose metabolism to insulin more than lipid metabolism (34). However, one year after surgery, those results suggested a decrease of lipid oxidation in the fasting state and under insulin infusion.

In conclusion, during the fasting state and during insulin infusion under conditions of euglycemic hyperinsulinemia, impairment of glucose oxidation and non-oxidative glucose disposal both contribute to the insulin resistance observed in obese class III patients. The bariatric surgery induced weight loss was associated with an improvement in insulin sensitivity dependent almost exclusively of non-oxidative glucose change. In addition, the high lipid oxidation did not change with weight loss, while insulin secretion was totally normalized.

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Table 1: Metabolic characteristics and *Clamp* results in Lean and Obese (studies I and II)

	Lean	Obese	
		Study I [p1]	*Study II* [p1, p2]
F. plasma glucose (mmol/l)	5.1 ± 0.1	5.1 ± 0.1	4.7 ± 0.1[a]
F. plasma insulin (pmol/l)	85 ± 5	332 ± 86[c]	68 ± 8[y]
SS. plasma insulin (pmol/l)	834 ± 55	915 ± 52	727 ± 57[x]
Insulin clearance (ml/min)	614 ± 35	1013 ± 74[b]	871 ± 54[b]
Serum uric acid – mg/dl	5.00 ± 0.37	7.80 ± 0,58[b]	5.33 ± 0.52[y]
Triglyceride - mgl/dl	69.2 ± 7.9	139.5 ± 11.5[b]	99.9 ± 13.2[x]
Total cholesterol - mg/dl	188.5 ± 11.2	175.2 ± 9.4	162.9 ± 5.4
HDL cholesterol - mgl/dl	43.2 ± 3.0	32.0 ± 1.8[a]	38.9 ± 2.7[b]

F – fasting period; SS – steady state insulin infusion period;

[p1] - OB II *vs*. CT – *Mann-Whitney test* –a = ≤ 0.05; b = p ≤ 0.001; c = p ≤ 0.0001;

[p2] - OB II *vs*. OB I – *Wilcoxon Signed rank* - x = p ≤ 0.05; y = p ≤ 0.001; z = ≤ 0.0001;

Table 2: Substrate utilization during fasting state and insulin infusion in Lean and Obese (studies I and II).

	Lean	Obese			
		Study I^{p1}	ANOVA$^{\#}$	*Study II*$^{p1, p2}$	ANOVA$^{\#\#}$
Fasting QR	0.79 ± 0.02	0.74 ± 0.01^{a}	0.001	0.71 ± 0.01^{b}	0.001
SS. QR	0.85 ± 0.02*	0.75 ± 0.01^{b}		0.77 ± 0.02^{a}*	
Fasting Protein Ox.	8.7 ± 0.9	5.6 ± 0.6^{b}	*ns*	4.1 ± 0.6^{b}	*ns*
SS. Protein Ox.	2.7 ± 3.7*	2.0 ± 1.3*		1.1 ± 1.2*	
Fasting Lipid Ox.	4.6 ± 0.6	6.1 ± 0.4^{a}	0.06	7.7 ± 0.4^{b}	0.01
SS. Lipid Ox.	4.2 ± 0.8	6.7 ± 0.6^{a}		6.9 ± 0.6^{a}	
Fasting Glucose Ox.	6.3 ± 2.1	1.7 ± 1.5	0.0009	-1.3 ± 1.2^{a}	0.03
SS. Glucose Ox.	15.6 ± 2.4*	4.3 ± 1.3*^{b}		5.9 ± 1.8*^{b}	
Non Ox. Gluc Disposal	35.9 ± 2.2	15.8 ± 1.9^{c}		29.6 ± 3.0^{x}	

QR – Respiratory Quotient; SS - Steady State;

^{p1} - OB I *or* OB II *vs*. CT – *Mann-Whitney test* – a = ≤ 0.05; b = p ≤ 0.001; c = p ≤ 0.0001;

^{p2} - OB II *vs*. OB I – *Wilcoxon signed rank* test - x = p ≤ 0.05;

***** - p ≤ 0.05 - SS period *vs.* fasting period - *Wilcoxon signed rank test;*

ANOVA for repeated measures – interaction group x time: p ≤ 0.05: $^{\#}$ - CT vs. Study I; $^{\#\#}$ - Studies I vs. II.

Table 3: Energy expenditure during fasting state and insulin infusion period in Lean and Obese (studies I and II).

	Lean	Obese	
		Study I^{p1}	*Study II*$^{p1, p2}$
REE (Kcal/day)	1449 ± 56	2262 ± 122^{c}	1808 ± 73^{a}
SSEE (Kcal/day)*	1496 ± 67	2426 ± 107^{c}	1942 ± 72^{b}
REE/ FFM (Kcal/FFM)	31.2 ± 1.3	28.3 ± 1.3	28.9 ± 0.6
SSEE/ FFM (Kcal/FFM)*	32.3 ± 1.6	30.3 ± 1.0	31.1 ± 0.6
REE/ BW (Kcal/kg BW)	24.0 ± 0.9	15.5 ± 0.9^{b}	19.6 ± 0.8^{b}
SSEE/ BW (Kcal/kg BW)*	24.8 ± 1.2	16.7 ± 0.9^{b}	21.1 ± 0.8^{b}

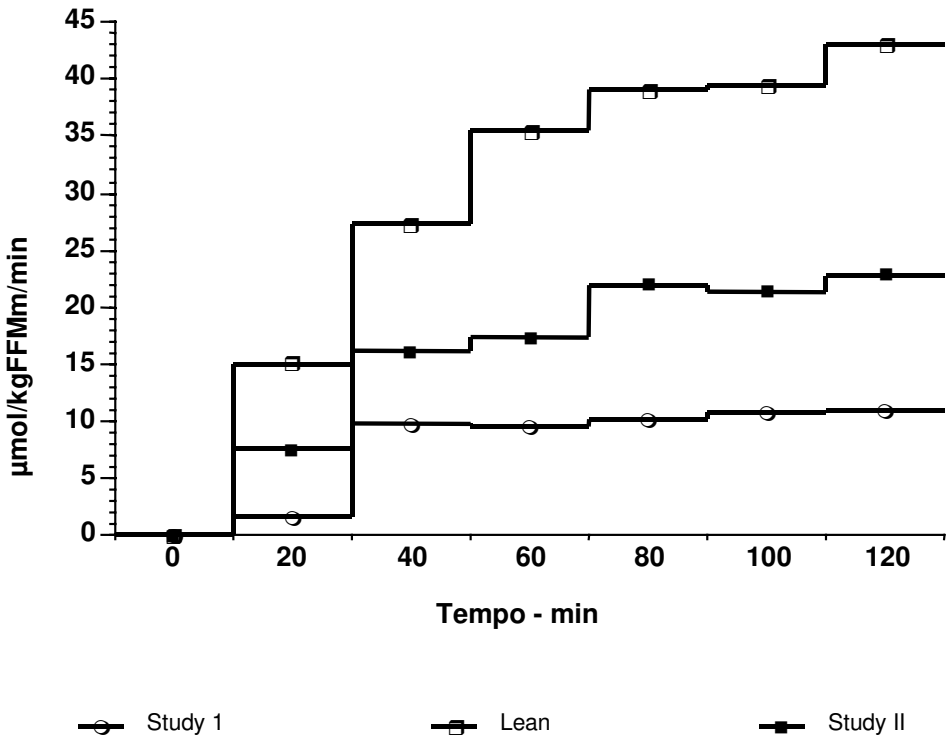
REE – resting energy expenditure; SSEE – steady state insulin infusion period energy expenditure;

^{p1} - OB I *or* OB II *vs*. CT – *Mann-Whitney test* –a = ≤ 0.05; b = p ≤ 0.001; c = p ≤ 0.0001;

^{p2} - OB II *vs*. OB I – *Wilcoxon signed rank* - x = p ≤ 0.05; y = p ≤ 0.001; z = ≤ 0.0001;

***** p ≤ 0.05 for SS period *vs.* fasting period – all groups - *Wilcoxon signed rank*

Figure 1: Glucose Utilization – M in Lean and Obese (studies I and II).



4. DISCUSSÃO GERAL

4.1. CIRURGIA E TRATAMENTO DA OBESIDADE:

O tratamento cirúrgico da obesidade tem sido considerado um método eficaz para a redução de peso. As medidas gerais de controle de peso, como restrição calórica, uso de medicamentos, mudanças comportamentais, entre outras, têm sido insuficientes para promover ou manter o emagrecimento nestes pacientes (MASON, 1997; WARDLE 1996).

Durante 14 meses, acompanhamos as modificações metabólicas, em um grupo de 13 pacientes obesos classe III, 9 mulheres e 4 homens, submetidos ao tratamento cirúrgico para a obesidade, no Hospital das Clínicas da Faculdade de Ciências Médicas da Universidade Estadual de Campinas (HC- FCM – UNICAMP). O primeiro estudo foi realizado antes da cirurgia, o segundo após um emagrecimento médio de 15 a 20 % do peso inicial e o terceiro após a estabilização do mesmo.

Os pacientes obesos foram operados e acompanhados pelo Grupo de Cirurgia da Obesidade do HC-FCM-UNICAMP e a técnica cirúrgica empregada foi a gastroplastia vertical com colocação de um anel de silástico, associada à derivação gastro-jejunal em Y de Roux, (técnica de Capella). Esta técnica tem se mostrado eficiente em promover o emagrecimento nos pacientes atendidos pelo serviço de cirurgia do HC-FCM-UNICAMP e a redução média de peso tem sido de 67,4 ± 13,4 % do peso corporal extra (GELONEZE 2002), semelhante às reduções médias de 60 a 85 % do peso corporal extra, previamente relatadas (SUGERMAN, et al. 1989; BROLIN, et al. 1994). A redução de peso está relacionada a alterações específicas da dinâmica e do funcionamento orgânico, alcançada em função do tipo específico de técnica utilizada segundo FLANCBAUM, 1999.

Todos os pacientes apresentaram glicemia normal de jejum, de acordo com os critérios revisados da *American Diabetes Association* (1997) e dois deles, após a realização do teste oral de tolerância à glicose, foram classificados como intolerantes à glicose. O grupo foi caracterizado antes da cirurgia por apresentar um IMC médio significativamente alto (56,7 ± 3,0 kg/m^{-2}), bem como o percentual de gordura, e foram encontradas as complicações metabólicas inerentes ao excesso de peso, tais como maiores níveis plasmáticos de ácido úrico, triglicérides e menor HDL. A cirurgia bariátrica proporcionou uma significativa redução de peso, mas no final do período de emagrecimento, os pacientes

ainda foram classificados como obesos (IMC médio de 33 kg/m^{-2}). A velocidade de emagrecimento foi maior nos primeiros 3 meses, quando os pacientes atingiram quase a metade do emagrecimento total observado na última avaliação.

Alguns estudos mostraram que o ritmo de emagrecimento foi maior nos primeiros 6 meses após a cirurgia. Depois deste período, ocorreu uma diminuição (FLICKINGER, et al. 1984) mas, em alguns casos, o emagrecimento persistiu por até 5 anos (SMITH, et al. 1995; KOLANOWSKY, 1997).

No presente estudo, tanto a massa magra quanto a adiposa foram reduzidas pelo emagrecimento, com maior efeito na segunda. Entretanto, esta avaliação foi realizada através da impedanciometria bioelétrica . Deve-se ressaltar que as modificações da hidratação da massa magra podem alterar o resultado e, em obesos após o emagrecimento, foi descrito aumento de água no compartimento extracelular (MAZARIEGOS et al. 1992; MARKEN LICHTENBELT e FOGELHOLM 1999).

4.2. SENSIBILIDADE À INSULINA:

Os pacientes foram avaliados na Unidade Metabólica do HC-FCM-UNICAMP e o método utilizado para medir a sensibilidade à insulina foi o *clamp* euglicêmico hiperinsulinêmico (DE FRONZO, et al. 1979). Antes de serem submetidos à cirurgia, os pacientes obesos apresentaram uma significativa resistência à ação da insulina, evidenciada pela captação total de glicose ("M") extremamente reduzida, quando comparados ao grupo controle. Estes resultados foram semelhantes aos encontrados por outros autores e mostram que a resistência à insulina é uma importante alteração metabólica encontrada em grandes obesos (MINGRONE, et al. 1999 e 1997; LETIEXHE, et al. 1995; JIMENEZ, et al. 1987). A sensibilidade à insulina foi calculada considerando-se apenas a taxa de infusão de glicose, corrigida pela massa magra, uma vez que a supressão da liberação da glicose endógena deveria estar suprimida nos níveis insulínicos atingidos em nossos estudos, independentemente do IMC, como mostrado por BONADONNA et al. (1990). No entanto, a quantidade de peso em excesso nos obesos estudados por este autor não foi tão grande quanto a observada em nossos pacientes e, nestes, não foi possível medir a produção endógena de glicose.

Após o emagrecimento, observou-se um significativo aumento da sensibilidade à insulina, mas os valores ainda foram inferiores aos encontrados no grupo de magros. É importante ressaltar que, após o fim do período de emagrecimento, caracterizado pela estabilização do peso, a redução ponderal foi de aproximadamente 40 %. Alguns autores têm sugerido que a redução do peso seria responsável pela melhora metabólica observada após a cirurgia. LETIEXHE et al. (1995) verificaram que após a normalização do peso em 8 mulheres obesas, não diabéticas, submetidas a gastroplastia vertical, ocorreu uma completa normalização da sensibilidade à insulina e da secreção de insulina. Estudos de LONG et al. (1994) mostraram que, após a redução de 50 % do peso corporal extra em pacientes submetidos à cirurgia de derivação gástrica, o risco de progressão do estado de intolerância à glicose para o diabetes diminuiu em 30 vezes. Segundo os autores, o emagrecimento foi o principal responsável pela modificação da sensibilidade à insulina. BURSTEIN et al. (1995) acompanharam um grupo de obesos submetidos à derivação gástrica durante um ano e observaram uma importante redução de peso, mas ao término do emagrecimento, os pacientes ainda apresentaram um IMC que os classificava como obesos (30,4±5,9 kg/m^2). No entanto, a sensibilidade à insulina, significativamente maior após a cirurgia, permaneceu inferior ao grupo controle e foi relacionada ao excesso de peso residual.

A melhora no metabolismo da glicose, após emagrecimento, é observada tanto em pacientes diabéticos tipo 2, quanto em pacientes intolerantes ou tolerantes à glicose. Reduções nas doses de medicamentos necessárias para o controle da hiperglicemia nos pacientes diabéticos são freqüentemente referidas e alguns destes trabalhos mostram a total suspensão da medicação em função do emagrecimento (SJOSTRÖN, 2000). PORIES et al. (1992) após acompanharem 479 pacientes, dentre eles, 101 pacientes diabéticos e 62 pacientes intolerantes à glicose, por um período de 10 anos observaram que apenas 5 % deles mantiveram um controle glicêmico inadequado.

Na cirurgia realizada, um dos mecanismos responsáveis pelo emagrecimento é a menor quantidade de energia ingerida durante as refeições (BRAY, et al. 1976; SUGERMAN, et al. 1992 b). Os pacientes ingerem uma média de 400 a 600 calorias dia e, logo após a cirurgia, aumentam para 800 a 1000. Por volta do sexto mês, ocorre uma

estabilização do consumo energético e é ingerida uma média diária de 1200 a 1400 calorias (BROLIN, et al. 1994). Outros fatores, relacionados à capacidade de absorção e à velocidade do trânsito intestinal, têm sido considerados importantes (KELLUM, et al. 1990). Além destes, foram descritas alterações específicas em vários hormônios gastro-intestinais, em pacientes submetidos às cirurgias para emagrecimento (SARSON, et al. 1981). A orientação nutricional aos pacientes acompanhados no presente estudo foi de aproximadamente 60% das calorias provenientes dos carboidratos complexos, 20% dos lipídios e 20% das proteínas, de acordo com as orientações do *National Institute of Health* (NIH, 1991). Não foi possível avaliar com maior precisão a aderência ao programa alimentar proposto.

Algumas técnicas cirúrgicas têm a capacidade de promover a redução específica de um determinado nutriente. A diversão bilio-pancreática é uma técnica que proporciona uma importante e significativa redução na absorção de gordura, por retardar e diminuir o contato entre a gordura e as secreções biliares e pancreáticas. Além disto, parece estar relacionada a uma rápida melhora da sensibilidade à insulina, por promover uma grande depleção da gordura intracelular, sensível à redução de gordura absorvida. MINGRONE et al. (1997) acompanharam um grupo de obesos diabéticos submetidos à diversão bilio-pancreática e mostraram uma significativa melhora da sensibilidade à insulina, após uma pequena redução de peso. Utilizando o *clamp* euglicêmico hiperinsulinêmico, comprovaram um importante aumento na utilização periférica de glicose, atribuída a uma significativa redução dos ácidos graxos não esterificados e triglicerídeos plasmáticos, concomitante à menor absorção de gordura observada após a cirurgia. A diminuição de lipídios foi considerada um evento importante para a melhora da sensibilidade à insulina em relação à perda de peso.

O aumento da oxidação de ácidos graxos livres foi associado à diminuição da oxidação de glicose por RANDLE e colaboradores na década de 60 e esta teoria ficou conhecida como ciclo de RANDLE. Segundo este autor, o aumento da oxidação dos ácidos graxos livres levaria a diminuição dos depósitos de NAD e ao acúmulo intracelular de acetyl-Coa e citrato, ambos inibidores das enzimas piruvatodesidrogenase (PDK) e a fosfofrutokinase (PFK), respectivamente. A redução das enzimas PDK e PFK altera a etapa

inicial do metabolismo da glicose provocando o acúmulo de glicose-6-fosfato, que por sua vez inibe a hexoquinase. A redução da hexoquinase diminui o transporte de glicose. Assim, haveria uma diminuição da oxidação de glicose pela inibição do ciclo de Krebes levando a uma diminuição da formação de glicogênio secundária ao menor transporte de glicose e alterações nas enzimas envolvidas no processo de formação de glicogênio (DEFRONZO, 1997).

Sugeriu-se que a captação de ácidos graxos livres pela musculatura esquelética é proporcional ao seu nível plasmático (KIENS, et al. 1989).

Alguns estudos têm mostrado uma relação inversa entre a sensibilidade à insulina e a quantidade de ácidos graxos livres presentes nos fosfolipídios da musculatura esquelética (BORKMAN, et al. 1993), a quantidade de triglicerídeos intramusculares (PAN, et al. 1997) e a quantidade de ácidos graxos saturados nos triglicérides musculares (MANCO, 2000).

O mecanismo envolvido na associação entre gordura intracelular e a redução da ação insulínica não está completamente esclarecido (PAN, 1997). A liberação de ácidos graxos livres de cadeia longa (*Long Chain Fatty Acyl-CoA,* LC-CoA) dos triglicerídeos intracelulares parece reduzir a ação da insulina sobre o metabolismo da glicose (OAKES, et al. 1997b; ELLIS, et al. 2000). O acúmulo de LC-CoA dentro das células parece estar relacionado a uma diminuição da atividade da enzima glicogênio sintase e a alterações no mecanismo de sinalização da insulina e transporte de glicose (OAKES, et al. 1997a). O Malonil CoA impede a entrada de LC-CoA dentro da mitocôndria, através de um efeito inibitório sobre a atividade da Carnitina-Palmitoil Transferase I, CPT I. Desta forma, o Malonil CoA exerceria um controle importante no tipo de substrato energético celular utilizado e na sensibilidade à insulina.

GRECO et al. (2002) avaliaram o impacto específico da diminuição da quantidade de lipídios intracelulares e da quantidade de lipídios interfibrilares presentes na musculatura esquelética, em relação à sensibilidade à insulina. Em seu estudo, foram acompanhados 20 obesos classe III, 10 submetidos à cirurgia de derivação bilio-pancreática e 10 mantidos sob tratamento clínico não cirúrgico para emagrecimento, todos tolerantes

normais à glicose. A redução de peso, após 6 meses de acompanhamento no grupo cirúrgico foi de 24 ± 5 % e no grupo não cirúrgico 9 ± 8 % do peso inicial. No grupo submetido à cirurgia, houve uma completa normalização da sensibilidade à insulina quando comparado aos indivíduos controle, entretanto estes pacientes ainda foram considerados obesos classe II. A quantidade de gordura perivascular, interfibrilar e principalmente a gordura intracelular apresentaram uma significativa redução em relação aos valores prévios à cirurgia, mas ainda foram maiores que os valores encontrados nos controles magros. A redução da gordura intracelular foi mais importante que a gordura extracelular ou a quantidade total de gordura no corpo para predizer o aumento da sensibilidade. No grupo submetido ao tratamento clínico, não ocorreram alterações na sensibilidade à insulina e na quantidade de gordura nos diferentes depósitos musculares. A significativa diminuição da absorção de lipídios pode ser um dos fatores responsáveis pelas diferenças entre os nossos resultados e os resultados apresentados por GRECO et al., 2002.

Um dos mecanismos descritos para diminuir o acúmulo de triglicerídeos tissulares (não no adiposo) é o aumento da leptina (UNGER, 2002). Este hormônio secretado pelos adipócitos pode estar relacionado também a esteatose hepática, um dos componentes da esteato-hepatite não alcoólica, NASH, e que é muito freqüente nos pacientes obesos graves (CHITURI et el., 2002). Nos pacientes avaliados por GRECO et al., 2002, a leptinemia diminui significativamente e foi associada à melhora da sensibilidade à insulina. É possível que, em nossos pacientes, este hormônio adipocitário também tenha diminuído consideravelmente, pois a redução da massa adiposa foi acentuada. Em acréscimo, a diminuição da leptinemia foi constatada por GELONEZE Et al., 2001, em um grupo composto, em parte, por alguns dos pacientes do presente trabalho.

A gordura abdominal tem sido relacionada à resistência à insulina (BONORA et al. 2000; 1992). A significativa redução da gordura abdominal nestes pacientes pode Ter sido um dos fatores relacionados ao aumento da sensibilidade à insulina.

Os depósitos abdominais se dividem em subcutâneo (anterior e posterior) (MISTRA, 1997) e visceral (DESPRÉS, et al. 1989) (omental, mesentérico e perirenal). O depósito visceral, também conhecido por depósito intra-abdominal, seria o responsável pela

liberação de uma grande quantidade de ácidos graxos livres diretamente na veia porta, aumentando o seu fluxo para o fígado (RODEN, et al. 1996). O aumento no sistema porta estaria relacionado com a resistência à insulina (FERRANNINI, et al. 1983).

O acúmulo de gordura intra-abdominal pode provocar compressões viscerais e dificultar o funcionamento dos pulmões, por deslocar o diafragma. Estudos de avaliação da composição corporal através da Ressonância Nuclear Magnética mostraram que, em grandes obesos (IMC $\geq$ 40), o volume de gordura intra abdominal pode ser maior que 6 litros (THOMAS, et al. 1998).

A medida de cintura, apesar de ser referida como um método indireto para a avaliação e quantificação da gordura abdominal, não pode ser considerada um parâmetro preciso nestes pacientes. O excesso de pele e o acúmulo de gordura abdominal, além de alterarem significativamente a estética corporal, diminuem a precisão das medições de cintura e quadril. Apesar destas limitações, o acompanhamento destes pacientes nos mostrou uma nítida redução da circunferência da cintura. Entretanto, não é possível definir qual a importância relativa da diminuição da gordura abdominal nestes pacientes e é possível que o grau de obesidade seja mais importante que a gordura visceral nos pacientes avaliados neste trabalho, como sugerido pelo estudo EGIR (FERRANNINI et al., 1997).

4.3. UTILIZAÇÃO DE SUBSTRATOS ENERGÉTICOS:

A oxidação de glicose basal nos pacientes obesos foi menor que a encontrada em indivíduos magros, mas não estatisticamente diferente. O emagrecimento não modificou este parâmetro, mas os valores foram significativamente menores, quando comparados aos magros. A infusão de insulina estimulou a oxidação de glicose em magros e obesos de forma diversa, sendo maior nos magros. Após o emagrecimento, a maior oxidação de glicose, comparada ao primeiro estudo, ainda foi menor do que a observada nos controles. A utilização não oxidativa da glicose foi medida durante o período de infusão de insulina e os valores encontrados no grupo de pacientes magros foram duas vezes maiores que os encontrados nos obesos. Esta foi a via metabólica mais influenciada pelo emagrecimento, uma vez que, no segundo estudo, os achados foram semelhantes aos dos magros.

Na situação de jejum, a oxidação de proteínas foi maior nos magros, quando comparada aos obesos antes ou após a redução do peso. A infusão de insulina proporcionou uma significativa redução da oxidação de proteínas em magros e obesos, tanto antes quanto após cirurgia bariátrica. O efeito supressivo da insulina também foi similar entre os grupos, portanto, não modificado pelo emagrecimento.

Em pessoas obesas ou diabéticas, as taxas de oxidação de lipídios e os níveis plasmáticos de ácidos graxos livres em jejum estão aumentados (FELBER et al., 1981). Nestes pacientes, no período pós absortivo, foram constatados aumentos da concentração de ácidos graxos livres e da oxidação de lipídios e uma reduzida capacidade de suprimir a oxidação de lipídios/ácidos graxos livres em resposta à insulina (BOGARDUS et al. 1984; LILLIOJA et al.1985). A resistência do tecido adiposo às ações da insulina e o aumento da lipólise em obesos resultam em maior liberação de ácidos graxos livres (REAVEN, 1995).

A oxidação basal de lipídios, no grupo dos obesos do presente estudo, foi maior que a encontrada no grupo de magros e persistiu após o emagrecimento. Durante o período de infusão de insulina, ocorreu uma discreta supressão da taxa de oxidação de lipídios nos magros, enquanto, nos pacientes obesos, ocorreu um discreto estímulo. Entretanto, após o emagrecimento, o comportamento do grupo sob o efeito da insulina foi diferente (estudo I *vs*. estudo II p= 0,001 – ANOVA) e a supressão da oxidação de lipídios foi semelhante à observada no grupo de controles.

É importante observar a correlação inversamente proporcional entre a oxidação de lipídios e glicose nos pacientes obesos, concordante com os resultados descridos por DEFRONZO (1997), que enfatizam a relação entre as duas vias metabólicas.

O aumento esperado do coeficiente respiratório, durante o *clamp*, foi observado nos indivíduos magros e nos obesos, apenas após o emagrecimento. A ausência de resposta na fase pré-cirúrgica demonstra a reduzida ação insulínica sobre o metabolismo. Portanto, os resultados obtidos através do *clamp* euglicêmico e da calorimetria apontam no mesmo sentido, ou seja, de uma maior resposta global à insulina após a redução do peso.

O gasto energético basal foi maior nos obesos, quando comparado aos magros ($p<0,05$), e não foi modificado pelo emagrecimento, permanecendo maior que os magros. Quando corrigido pela massa magra, não houve diferença estatística entre magros e obesos pré ou pós-cirurgia bariátrica. Em todas as etapas do estudo, a infusão de insulina aumentou o gasto metabólico em obesos, assim como nos controles. A constância do gasto energético, por unidade de massa magra, pode sugerir que, medidas que a preservem sejam úteis na manutenção do gasto energético, diminuindo o risco de recuperar o peso perdido.

4.4. SECREÇÃO DE INSULINA

A redução dos níveis plasmáticos de insulina foi outro importante sinal da melhora metabólica observada em nosso grupo de estudo. A insulinemia de jejum nos pacientes obesos foi significativamente maior que a encontrada no grupo dos magros e semelhante após o emagrecimento. A hiperinsulinemia é considerada um fator de risco isolado para doenças cardiovasculares.

Existem evidências de que, a hiperinsulinemia em obesos seria uma resposta compensatória à menor ação da insulina. Os mecanismos responsáveis pelo aumento das concentrações de insulina ainda não estão totalmente esclarecidos. Em obesos, tem sido atribuído a alterações do clearance hepático de insulina (ROSSELL, et al. 1983; MEISTAS, et al. 1983) e a estímulos diretos na célula beta (BONORA, et al. 1984). Entretanto, as alterações do clearance não foram confirmadas por outros autores (POLONSKY et al., 1988; MUSCELLI et al., 1997) e, no presente estudo, seguramente não contribuíram para a hiperinsulinemia, pois era discretamente aumentado nos obesos, comparado ao grupo controle, e não mudou com o emagrecimento.

JIMENEZ et al. (1987) acompanharam um grupo de pacientes obesos, (IMC ~ 47 kg/m^2), submetidos à gastroplastia vertical com bandagem e, através do *clamp* hiperglicêmico, comprovaram a normalização da sensibilidade à insulina ao final do período de emagrecimento e estabilização do peso (IMC ~ 31 kg/m^2). O aumento do clearance metabólico de insulina foi considerado o evento inicial para a redução das taxas

de insulina circulante, enquanto a diminuição da secreção de insulina só foi observada no final da fase de emagrecimento.

Na fase pré-operatória, os pacientes estudados na Unidade Metabólica do HC-FCM-UNICAMP apresentaram uma diminuição da capacidade de auto inibição da secreção de insulina (apenas 11 % comparada a cerca de 40% nos controles). As concentrações plasmáticas de peptídeo C foram medidas durante as fases do *clamp* (jejum e infusão de insulina) para avaliar a secreção hormonal. As variações plasmáticas do peptídeo C são utilizadas devido a sua secreção molar equivalente à da insulina, insignificante extração hepática e pelo clearance semelhante em indivíduos obesos e magros (POLONSKY, et al. 1983; 1988). Após a redução de peso, a inibição da secreção de peptídeo-C/insulina foi mais acentuada, semelhante àquela observada nos controles.

DEFRONZO et al. (1981) demonstraram redução de 53% na secreção de insulina, em indivíduos magros, com insulinemia de 25 uU/ml, sugerindo que a inibição ocorre em baixas doses de infusão de insulina. Não foram observados efeitos adicionais, quando os níveis plasmáticos de insulina foram aumentados. Este achado sugere que os valores de insulina usados no nosso estudo deveriam produzir uma resposta inibitória máxima, o que não ocorreu.

Os mecanismos, pelos quais um aumento na concentração de insulina inibe sua própria secreção, não estão completamente esclarecidos. Os resultados deste estudo, relativos à auto-inibição da secreção de insulina, sugerem que um dos mecanismos envolvidos na menor resposta dos obesos seja uma resistência primária ou secundária da celula β a esta ação. Desta forma, a inibição da secreção de insulina foi diretamente proporcional à sensibilidade à insulina nos indivíduos magros. Por outro lado, sugerem que a alterada resposta dos obesos à infusão de insulina é reversível e acompanha o emagrecimento, pois foi observada uma correlação com o IMC e a massa magra.

A secreção basal de peptídeo C foi relacionada principalmente com a sensibilidade à insulina, quando foram incluídos os valores de IMC, cintura e M, no modelo de regressão linear usado, em concordância com a premissa de que a hiperinsulinemia é em boa parte secundária à resistência à insulina.

Resultados de estudos anteriores sugerem que a inibição da secreção de insulina pancreática pela insulina pode ser mediada neuronalmente (BODEN, et al. 1993). Em adição, nos indivíduos magros, a insulina ativa o sistema simpático (ANDERSON, et al. 1991) e causa uma mudança no balanço do sistema nervoso autônomo (SCHERRER, 1994), com um aumento na relação simpático/parassimpático (MUSCELLI et al., 1998). Pacientes obesos apresentam, freqüentemente, um tônus simpático em jejum maior que os magros, mas a resposta à infusão de insulina tem sido menor ou ausente. (MUSCELLI et al., 1998; SCHERRER, 1994). Desta forma, durante o *clamp*, uma ação inibitória simpática sobre a secreção de insulina deveria ser menos marcada que nos indivíduos magros. O emagrecimento é acompanhado de maior resposta simpática durante o *clamp* (ENDIM

et al., 2001), o que seria compatível com a maior auto-inibição pela insulina, verificada no presente estudo.

5. CONCLUSÃO GERAL

O presente estudo nos permitiu constatar a grande eficácia da cirurgia de gastroplastia vertical com bandagem e derivação gastro-jejunal em reduzir o peso corporal e melhorar a composição corporal. O emagrecimento alcançado foi associado à normalização da secreção de insulina e ao aumento da capacidade da insulina em inibir sua própria secreção. Entretanto, a sensibilidade à insulina no período de seguimento melhorou significativamente, mas continuou menor que aquela observada nos indivíduos magros.

Quanto às diferentes ações da insulina, verificamos que foi restabelecido o seu efeito sobre a utilização não oxidativa da glicose. Porém, apenas um discreto efeito foi verificado na via oxidativa. O aumento da oxidação basal de lipídios e a ausência de resposta à insulina não foram modificados significativamente, pelo emagrecimento.

Portanto, confirmamos que as modificações relacionadas ao emagrecimento são desproporcionais entre si e ocorrem em tempos distintos.

Estes achados seguramente não invalidam o procedimento, pois vários outros benefícios não foram relatados no presente trabalho, incluindo a importante melhora da qualidade de vida e a auto-estima observadas após o emagrecimento. Também não foram aqui avaliadas as complicações eventualmente encontradas.

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