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Young, low-birth-weight men are not more susceptible to the diabetogenic effects of a prolonged free fatty acid exposure than matched controls☆

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Abstract

Low birth weight (LBW) is associated with increased risk of developing type 2 diabetes later in life. Progression from normal to impaired glucose tolerance and overt diabetes may depend, to some extent, on elevation of plasma free fatty acids (FFAs). We undertook this study to elucidate whether a prolonged physiological lipid load could unmask or augment existing metabolic defects in otherwise healthy young LBW subjects. Forty 19-year-old men (LBW [n = 20], controls [normal birth weight, NBW] [n = 20]) without a family history of diabetes underwent an intravenous glucose tolerance test (0.3 g kg^{-1}), followed by 2-step hyperinsulinemic-euglycemic clamps (2 × 120 minutes: 10 and 40 mU m^{-2} min^{-1}) in combination with [3-^{3}H]-glucose and indirect calorimetry. The tests were preceded, in randomized order, by a 24-hour continuous intralipid (20%, 0.4 mg mL^{-1} h^{-1}) or saline infusion. Estimates of cellular glucose metabolism were obtained and a disposition index calculated. Clamp FFA concentrations were 4- to ten-fold higher during lipid infusion. Both groups experienced a similar decrease in insulin-stimulated glucose disposal in response to lipid infusion (~15%; $P < .05$), which was mainly accounted for by reduced glucose oxidation (~30%; $P < .001$). Glycolysis, glucose storage, and glucose production were not significantly altered by lipid infusion. Nevertheless, the LBW group had significantly lower insulin-stimulated glycolysis during lipid infusion (~27%; $P < .05$) than the NBW group. An appropriate increase in insulin secretion matched the decline in insulin sensitivity in both groups. A 24-hour low-grade intralipid infusion has similar effects on whole-body glucose metabolism and first-phase insulin secretion in 19-year-old, healthy, lean, LBW men with normal glucose tolerance and in NBW controls. We reproduced our previous finding of lower insulin-stimulated glycolysis in this population.

1. Introduction

Based on their original observations in 2 independent British populations [1,2], Barker and Hales [3] pioneered the idea that abnormal glucose tolerance (impaired glucose tolerance [IGT] and type 2 diabetes) may arise as a result of "programming" early in life: as an adaptation to undernutrition in fetal life, permanent metabolic and endocrine changes occur which would be beneficial if nutrition remained scarce after birth. However, with abundant postnatal nutrition these changes predispose to abnormal glucose tolerance and obesity. A number of animal [4] and human studies [5-10] have confirmed an association between an early adverse environment and obesity later in life. Moreover, it appears that low birth weight (LBW) and current body mass index (BMI) interact in a multiplicative fashion, such that the highest risks of abnormal glucose tolerance were in those who were small at birth but became overweight adults [6,11-14].

Obesity represents an expansion of adipose tissue mass, and one explanation for the obesity-related effects on carbohydrate metabolism is the production of adipose

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tissue–derived products [15]. Although several candidates, including hormones such as leptin, adiponectin, and cytokines such as TNF-α and IL-6, have been proposed to mediate these effects, substantial evidence still favors free fatty acids (FFAs) as a major player (reviewed in Refs. [16,17]). In summary, when the fat depot is expanded, plasma FFA becomes elevated. Increased tissue availability of FFA, and as a result intracellular lipid accumulation, has been shown to impair the ability of insulin to stimulate glucose uptake into skeletal muscle, possibly by altering insulin signaling [18-20] and by influencing expression of genes involved in intracellular glucose and lipid metabolism [21]. In addition, elevated FFA was shown to impair insulin suppression of hepatic glucose output [22,23], and insulin secretion from pancreatic β cells was reduced in some but not all studies (reviewed in Refs. [24,25]). High-fat feeding was recently shown to accelerate and augment the impaired glucoregulation in protein-restricted rat [26,27], an animal model of intrauterine growth retardation. Similar intervention studies are lacking in humans.

We recently reported that 19-year-old glucose-tolerant men with birth weights below the 10th percentile for gestational age have significantly lower insulin-stimulated glycolysis [28] and reduced forearm glucose uptake [29] than matched normal birth weight (NBW) controls, despite perfectly normal whole-body glucose disposal.

In parallel with the findings in the protein-restricted rat, we hypothesized that a prolonged physiological lipid load might aggravate or unmask underlying metabolic defects in these human subjects with low birth weight. Data and discussion of the effects of lipid infusion on glucose metabolism in the healthy control subjects have been published previously in a separate paper [30] and will not be described in much detail here.

2. Subjects and methods

2.1. Subjects characteristics

Forty healthy male volunteers born at term in 1980 were recruited from the Danish Medical Birth Registry according to birth weight (BW) (Table 1). Twenty subjects (LBW) had BW below the 10th percentile (mean BW, 2702 ± 45 g), and 20 subjects (NBW) had BW in the upper normal range (50th-75th percentile) (mean BW, 3801 ± 22 g). The demographic and metabolic data from the control (saline) experiment are described in more detail in Ref. [28]. In summary, all had normal glucose tolerance, and subjects with a personal or family history of diabetes were excluded. There were no significant differences in current weight, BMI, waist-to-hip (W/H) ratio, total fat mass, and lean body mass as determined by dual-energy x-ray absorptiometry (DXA) scan, $V_{O_{2max}}$, systolic and diastolic blood pressure, or plasma lipids between the groups. There were 2 light smokers (5-10 cigarettes) in each group. Nevertheless, the LBW subjects had slightly higher fasting (5.6 ± 0.1 vs 5.4 ± 0.1 mmol L^{-1}; $P < .05$) and 2-hour postload plasma glucose (5.9 ± 0.9 vs 5.3 ± 0.2 mmol L^{-1}; $P = .075$) during an oral glucose tolerance test (done on a separate day), as well as lower fasting plasma high-density lipoprotein cholesterol (1.16 ± 0.06 vs 1.31 ± 0.05 mmol L^{-1}; $P < .05$). Informed written consent was obtained from all study subjects before participation. The protocol was approved by the regional ethical committee, and all procedures were performed in accordance with the guidelines of the Declaration of Helsinki.

Table 1
Subjects characteristics

	LBW (n = 20)	NBW (n = 20)	P
Birth weight (g)	2702 ± 202.0	3800.5 ± 98.7	<.0001
Weight (kg)	73.6 ± 8.5	74.7 ± 13.1	NS
Height (cm)	178.5 ± 4.0	181.7 ± 4.8	.03
BMI (kg m^{-2})	23.1 ± 2.7	22.6 ± 3.6	NS
W/H ratio	0.82 ± 0.04	0.81 ± 0.06	NS
Systolic BP (mm Hg)	111.3 ± 11.7	114.8 ± 11.5	NS
Diastolic BP (mm Hg)	65.5 ± 8.9	67.8 ± 9.8	NS
F-P-TG (mmol L^{-1})	1.09 ± 0.66	0.92 ± 0.30	NS
F-P-Cholesterol, total (mmol L^{-1})	4.03 ± 0.70	3.88 ± 0.56	NS
F-P-LDL (mmol L^{-1})	2.37 ± 0.56	2.16 ± 0.44	NS
F-P-HDL (mmol L^{-1})	1.16 ± 0.26	1.31 ± 0.21	.05
Total fat $mass_{DXA}$ (kg)	15.6 ± 6.8	15.6 ± 6.9	NS
Fat free $mass_{DXA}$ (kg)	54.9 ± 4.3	56.9 ± 7.3	NS
$V_{O_{2max}}$ (L min^{-1})	3.4 ± 0.4	3.5 ± 0.7	NS

Data are mean ± SD. BP indicates blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

2.2. Experimental protocol

All subjects were studied on 2 occasions (3-5 weeks apart), preceded in randomized order by a 24-hour intralipid (LIP) or saline (SAL) infusion (Fig. 1). No changes in diet, weight, or lifestyle were recorded from the time of recruitment until the completion of both studies. For at least 48 hours before the experiment, the participants were instructed to consume a diet rich in carbohydrate and abstain from strenuous physical activity and alcohol. The study subjects reported to the laboratory at 8:00 AM after a 10-hour overnight fast (day −1, SAL or LIP). A polyethylene catheter was placed in the antecubital vein for test infusions (LIP: intralipid 20%, 0.4 mL kg^{-1} h^{-1}; heparin 200 U [bolus], 0.2 U kg^{-1} h^{-1}; or SAL: NaCl 9 g L^{-1}, 0.4 mL kg^{-1} h^{-1}). The test substances were infused for 24 hours and continued throughout the intravenous glucose tolerance test (IVGTT) and clamp studies (in total 30½ hours). A second catheter was placed in a dorsal hand vein of the contralateral arm for blood sampling. The hand was placed in a heated Plexiglas box to ensure arterialization of the venous sample. Standardized meals were served at +15 minutes (breakfast), +3 hours 15 minutes (lunch), +9 hours (dinner), +12 hours 30 minutes (sandwich), and a standardized 15-minute light exercise on a bicycle was

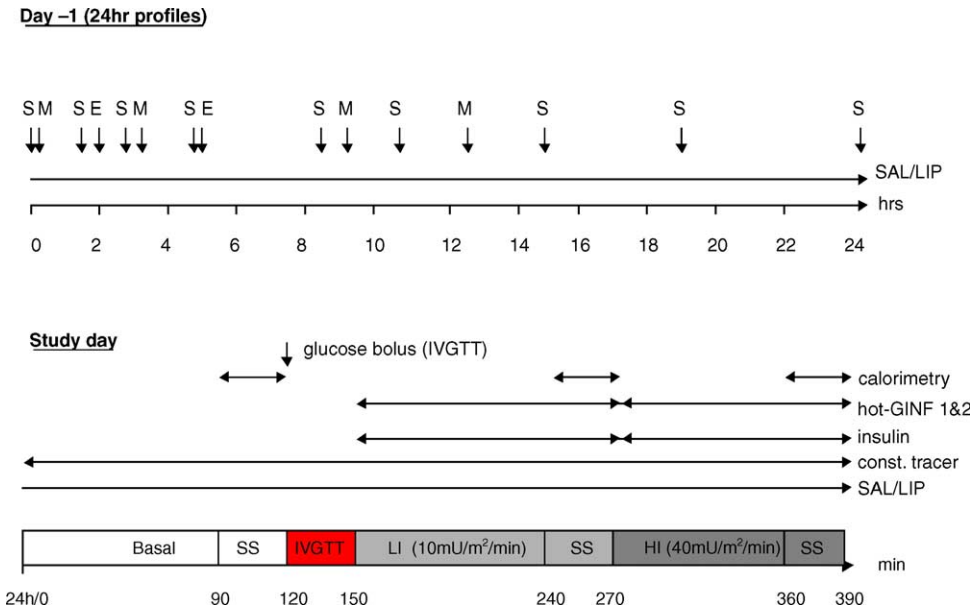

Fig. 1. Study design.

performed at +2 and +5 hours after initiation of the study. The following day (study day, SAL or LIP), after a 10-hour fast, a primed-continuous infusion of [3-^{3}H]-tritiated glucose (bolus, 10.9 μCi, 0.109 μCi min^{-1}) was initiated at 0 hours and sustained throughout the 2-hour basal period (0-120 minutes), 30-minute IVGTT (+120-150 minutes), and clamp studies (+150-390 minutes). A 1-minute intravenous glucose bolus (0.3 g kg^{-1} body weight) was given immediately after the 2-hour basal period (+120 minutes). Blood samples for analysis of glucose, insulin, and C-peptide were drawn at 0, 2, 4, 6, 8, 10, 15, 20, and 30 minutes. A primed-continuous insulin infusion (square wave bolus, 2 U, 10 mU m^{-2} min^{-1}) was begun at +150 minutes, continued for 120 minutes during the low physiological insulin clamp (LI, 150-270 minutes), and was raised to 40 mU m^{-2} min^{-1} during the 120-minute high physiological insulin clamp (HI, 270-390 minutes). Steady state was defined as the last 30 minutes of each 2-hour basal, LI-, and HI clamp period, respectively, when tracer equilibrium (ie, constant specific activity) was anticipated. Indirect calorimetry was performed during all 3 steady-state periods using a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as previously described [31]. Variable infusion of "cold" glucose (180 g L^{-1}) enriched with tritiated glucose (HOT-GINF) (LI, 13.75 μCi/500 mL; HI, 55 μCi/500 mL) maintained euglycemia during insulin infusion. Plasma glucose concentration was monitored every 5 to 10 minutes during basal and insulin-stimulated steady-state periods. Blood samples for analysis of insulin, C-peptide, glucagon, and FFAs were drawn every 30 minutes, tritiated glucose/water every 10 minutes during steady-state periods, and every 30 minutes for the rest of the study period. This clamp, also known as the Botnia clamp, has been validated against a regular euglycemic clamp without prior IVGTT, showing a high degree of reproducibility of the measurements obtained in the 2 clamps [32].

2.3. Analytical procedures

Plasma glucose was measured at bedside by an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, Calif). Plasma insulin, C-peptide, and glucagon blood samples were centrifuged immediately at 4°C and stored at −80°C for later analysis. Plasma insulin and C-peptide concentrations were determined by 1235 AutoDELPHIA automatic immunoassay system (Wallac Oy, Turku, Finland). The plasma insulin assay had a detection limit of approximately 3 pmol L^{-1}. Cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32-33 split proinsulin, and 66% with 64-65 split proinsulin, intra-assay coefficient of variation 4.5% and interassay variation 7%. Detection limit of the C-peptide assay was 5 pmol L^{-1}. Cross-reactivity with intact proinsulin was 51%, 35% with 32-33 split proinsulin, and 92% with 64-64 split proinsulin (no detectable cross-reactivity with insulin), intra-assay variation 5% and interassay variation 8%. The glucagon assay [33] is directed against the COOH terminus of the glucagon molecule (antibody code no. 4305) and measures glucagon of mainly pancreatic origin. Plasma was extracted with ethanol (final concentration, 70% vol/vol) before analysis. Detection limit and intra-assay coefficient of variation of the assay used were 1 pmol L^{-1} and <6%, respectively. Tritiated glucose and water were measured as described by Hother-Nielsen et al [34]. Plasma FFAs were quantified by an enzymatic colorimetric method (Wako, Neuss, Germany). Lipoprotein lipase inhibitor was not

added, and hence the true FFA concentrations may be slightly lower. Plasma lactate was quantified on an automatic lactate analyzer (YSI 23AM; Yellow Springs International, Yellow Springs, Ohio), whereas concentrations of alanine, D-β-hydroxybutyrate, and glycerol were measured by a fluorometric method.

2.4. Calculations

2.4.1. Basal and insulin-stimulated glucose turnover rates

Appearance rates (Ra), disposal rates (Rd), and endogenous glucose production (EGP) rates were calculated at 10-minute intervals during steady state, using Steele's non–steady-state equations [35]. In the calculations, distribution volume of glucose was set to 200 mL per kilogram of body weight and the pool fraction 0.65. Rates of whole-body glycolysis (exogenous glycolytic flux [GF]) were estimated from the increment per unit time in tritiated water ([cpm mL^{-1} min^{-1}] × total body water mass [mL]/[3-^{3}H] glucose specific activity [cpm $mmol^{-1}$]) [36]. Plasma water was estimated as 93% of total plasma volume, and total body water mass was assumed to be 65% of the body mass [37]. Endogenous glucose production was calculated by subtracting the exogenous glucose infusion rate from the rate of appearance of glucose; exogenous glucose storage (EGS) as Rd minus GF. Glucose turnover rates were expressed as mg kg FFM^{-1} min^{-1} and presented throughout the paper as mean values of the 30-minute steady-state periods. In addition, a sensitivity index (Si_{Rd}) was calculated. In contrast to the original index [38] obtained at dynamic conditions during a frequently sampled IVGTT, the sensitivity index used in this paper represents the net increase in glucose disposal above basal per unit change in plasma insulin concentration during hyperinsulinemia (40 mU m^{-2} min^{-1}) adjusted for mean steady-state glucose concentrations $\{(Rd_{40\ mU}-Rd_{basal})/[(insulin_{40\ mU}-insulin_{basal}) \times gluc_{40\ mU}]\}$(mg × kg FFM^{-1} × min^{-1} × pmol L^{-1} $insulin^{-1}$ × mmol L^{-1} $gluc^{-1}$) during the clamp (ie, tracer, glucose, and insulin all at steady state). Lipid oxidation rates were derived from indirect calorimetry, as described in Ref. [31].

2.4.2. Intravenous glucose tolerance test

Prehepatic insulin secretion rates (ISRs) were calculated by deconvolution of peripheral C-peptide concentrations, using a 2-compartment model of C-peptide kinetics [39,40] and population-based C-peptide kinetic parameters [41]. The population-based parameters are derived from analysis of a large number of individual kinetic parameters allowing adjustment for clinical status (in this case normal), age, and body surface [41]. Calculations were based on the assumption that only hepatic clearance of insulin but *not* C-peptide was affected by intralipid infusion. (The software was kindly provided by Mr Aage Vølund, Department of Statistics, Novo Nordisk A/S, Denmark.) Total and incremental area under the curve (AUC) for glucose (mmol L^{-1} × min), insulin (pmol L^{-1} × min), C-peptide (pmol L^{-1} × min), and ISR (pmol L^{-1} × kg^{-1}) were calculated by means of the trapezoidal rule from 0 to 10 minutes during IVGTT (only incremental values in table). The disposition index (DI) was calculated as the product between Si_{Rd} and incremental AUC_{ISR} (mg glucose × kg FFM^{-1} × min^{-1} × pmol L^{-1} insulin × kg^{-1}).

2.4.3. Insulin clearance

On day −1 (SAL or LIP), a measure of insulin clearance was obtained by calculation of the ratio between the area under the C-peptide curve and the area under the insulin curve (pmol L^{-1} C-peptide × pmol L^{-1} $insulin^{-1}$), whereas on the study day (SAL or LIP), insulin clearance was calculated as the mean steady-state C-peptide concentration divided by the mean steady-state insulin concentration (basal, pmol L^{-1} C-peptide × pmol L^{-1} insulin), or insulin infusion rate (LI, 10 mU m^{-2} min^{-1}; HI, 40 mU m^{-2} min^{-1}) divided by the mean steady-state plasma insulin concentration (L × m^{-2} × min^{-1}).

2.4.4. Statistical analyses

Nonparametrical Wilcoxon test for paired data, Mann-Whitney for unpaired data, and Friedman's test (analysis of variance for repeated measurements, nonparametrical data) were used in the data analysis. Calculations were performed with Instat software (Instat Statistical Package, GraphPad Inc, San Diego, Calif). Data on subject characteristics are presented as mean ± SD; all other data as uncorrected mean ± SEM. However, the data did not change if corrected for basal values (glucose or substrate fluxes), or for potential confounders such as BMI, W/H ratio, or fat mass data. $P < .05$ was considered significant in 2-tailed analysis.

3. Results

3.1. Twenty-four–hour profiles

The 2 groups responded similarly to intralipid infusion (LIP vs SAL) with an increase in AUC for glucose, insulin, C-peptide, and FFA, whereas AUC for glucagon was unchanged, and insulin clearance decreased (incremental AUC values not different between groups; data not shown) (Table 2).

3.2. Plasma glucose, insulin, C-peptide, glucagon, FFA, glycerol, β-hydroxybutyrate, lactate, and alanine concentrations during basal and insulin-stimulated steady-state periods

Lipid infusion resulted in similar basal and insulin-stimulated FFA concentrations between the groups (ie, FFA clearance was not different between groups) (Table 3). It is worth noting though that despite an approximately 2-fold increase at all time points during day 1, plasma FFA approached the same level in the SAL and LIP experiment toward the end of the 24-hour period in both groups and was

Table 2
Twenty-four–hour profiles

	LBW		NBW	
	SAL	LIP	SAL	LIP
$AUC_{glucose}$	152.9 ± 2.1	159.0 ± 1.8**	153.8 ± 2.0	159.0 ± 1.9
$AUC_{insulin}$	2228 ± 213	2875 ± 343***	2011 ± 129	2652 ± 179***
$AUC_{C\text{-}peptide}$	24215 ± 1594	28081 ± 2092**	22104 ± 1102	25608 ± 1192**
$AUC_{glucagon}$	191.2 ± 14.2	194.2 ± 9.1	189.1 ± 13.8	181.0 ± 10.1
AUC_{FFA}	7.3 ± 0.6	14.6 ± 1.3****	7.6 ± 0.4	13.7 ± 1.2****
Insulin clearance	11.6 ± 0.7	10.7 ± 0.6*	11.5 ± 0.6	10.1 ± 0.5**

SAL vs LIP: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < 0.0001$. LBW vs NBW, all NS.
For units please see Subjects and Methods section.

not significantly higher in the LIP study at basal steady state. Plasma glucose was unchanged in both groups at basal and HI, but increased slightly in both groups at LI. The LBW group tended to have higher basal and, in particular, clamp plasma insulin concentrations than controls during intralipid infusion (HI-LIP, 353.5 ± 17.4 vs 315.1 ± 8.1 pmol L^{-1}; $P = .09$). Furthermore, clamp C-peptide concentrations increased significantly during lipid infusion in the LBW group but not in NBW controls. In contrast, clamp plasma glucagon concentrations increased significantly in the NBW group (LI-LIP vs LI-SAL; $P < .01$; HI-LIP vs HI-SAL; $P < .05$) and insulin/glucagon ratio decreased (LI-LIP vs LI-SAL; $P < .05$; HI-LIP vs HI-SAL; $P < .05$); no such change was observed in the LBW group. Plasma glycerol, lactate, alanine, and β-hydroxybutyrate responses were similar in the groups (data not shown).

3.3. Glucose fluxes during basal and insulin-stimulated steady-state periods

Similar responses to intralipid infusion (LIP vs SAL) were observed in the 2 groups with regard to basal and insulin-stimulated glucose disposal (Rd: basal and LI, no change; HI, ↓; $P < .05$), glucose oxidation (GOX: basal, no change; LI and HI, ↓; $P < .01$), glycolytic flux (GF: basal, LI, and HI: no change), endogenous glucose production (EGP: basal and HI, no change), glucose storage (EGS:

Table 3
Clamp data I

		LBW		NBW	
		SAL	LIP	SAL	LIP
P-Glucose (mmol L^{-1})	Basal	5.44 ± 0.05	5.54 ± 0.08	5.41 ± 0.05	5.52 ± 0.06
	LI	5.07 ± 0.04	5.21 ± 0.05*	5.03 ± 0.04	5.17 ± 0.03**
	HI	5.08 ± 0.04	5.07 ± 0.05	4.98 ± 0.05	5.05 ± 0.04
P-Insulin (pmol L^{-1})	Basal	44.4 ± 6.2	63.9 ± 11.2****	41.6 ± 3.14	52.5 ± 3.9****
	LI	128.0 ± 9.5	140.9 ± 13.0**	109.1 ± 3.6	120.9 ± 3.5**
	HI	340.0 ± 15.1	353.5 ± 17.4	325.1 ± 7.3	315.1 ± 8.1
P-C-peptide (pmol L^{-1})	Basal	528.7 ± 45.5	642.5 ± 62.9***	479.8 ± 27.9	570.0 ± 39.8**
	LI	586.9 ± 67.1	656.4 ± 86.3*	504.4 ± 30.7	529.2 ± 37.8
	HI	338.7 ± 33.1	411.7 ± 61.4*	303.0 ± 22.9	320.2 ± 25.6
P-Glucagon (pmol L^{-1})	Basal	7.8 ± 0.8	8.5 ± 0.7	7.9 ± 0.5	8.0 ± 0.6
	LI	6.4 ± 0.4	6.9 ± 0.5	5.5 ± 0.4	6.9 ± 0.6**
	HI	4.8 ± 0.6	5.6 ± 0.5	4.0 ± 0.4	5.0 ± 0.5*
P-FFA (mmol L^{-1})	Basal	0.47 ± 0.04	0.57 ± 0.05	0.56 ± 0.04	0.59 ± 0.05
	LI	0.09 ± 0.02	0.45 ± 0.05***	0.12 ± 0.02	0.44 ± 0.04****
	HI	0.05 ± 0.03	0.37 ± 0.05***	0.02 ± 0.02	0.33 ± 0.03****
P-Glycerol (mmol L^{-1})	Basal	51.8 ± 3.7	97.1 ± 5.2****	67.3 ± 4.8§	109.2 ± 8.6****
	LI	21.8 ± 3.0	103.0 ± 4.9****	29.9 ± 3.2	104.0 ± 5.0****
	HI	19.7 ± 3.5	93.4 ± 4.9****	19.5 ± 2.4	94.2 ± 4.8****
Specific activity (cpm mg^{-1})	Basal	261.3 ± 4.9	250.9 ± 6.9	237.7 ± 7.5	230.3 ± 11.8
	LI	224.5 ± 8.4	203.9 ± 6.7	213.6 ± 7.4	196.1 ± 7.6*
	HI	300.3 ± 9.0	295.7 ± 6.2	278.6 ± 6.4	274.0 ± 7.2§
Insulin clearance	Basal	13.72 ± 1.37	11.46 ± 0.63*	12.41 ± 0.96	11.31 ± 0.71*
	LI	0.50 ± 0.03	0.47 ± 0.03*	0.56 ± 0.02	0.50 ± 0.02**
	HI	0.73 ± 0.03	0.71 ± 0.03	0.75 ± 0.02	0.77 ± 0.02
Insulin/glucagon	Basal	5.89 ± 0.52	7.32 ± 0.77**	5.86 ± 0.69	6.81 ± 0.66**
	LI	20.49 ± 1.04	20.76 ± 1.04	22.19 ± 2.22	18.27 ± 1.24*
	HI	84.83 ± 8.89	70.53 ± 5.48	108.85 ± 16.58	75.69 ± 10.47*

SAL vs LIP: *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$; LBW vs NBW: §$P < .05$.

Table 4
Clamp data II

		LBW		NBW	
		SAL	LIP	SAL	LIP
Rd (mg kg FFM^{-1} min^{-1})	Basal	2.87 ± 0.11	3.13 ± 0.26	2.99 ± 0.09	3.29 ± 0.35
	LI	4.01 ± 0.25	4.10 ± 0.21	3.84 ± 0.15	4.01 ± 0.19
	HI	11.11 ± 0.68	9.52 ± 0.67**	11.86 ± 0.68	10.42 ± 0.77*
GOX (mg kg FFM^{-1} min^{-1})	Basal	2.44 ± 0.25	2.05 ± 0.13	1.98 ± 0.15	2.17 ± 0.18
	LI	2.72 ± 0.12	2.30 ± 0.12**	2.64 ± 0.17	2.36 ± 0.16**
	HI	4.79 ± 0.18	3.44 ± 0.16***	4.82 ± 0.18	3.68 ± 0.26**
GF (mg kg FFM^{-1} min^{-1})	Basal	2.35 ± 0.51	2.83 ± 0.66	2.54 ± 0.39	3.08 ± 0.50
	LI	3.09 ± 0.40	3.72 ± 0.40	2.92 ± 0.45	3.68 ± 0.41
	HI	3.94 ± 0.58	3.58 ± 0.59	5.38 ± 0.58§	4.91 ± 0.43§
EGP (mg kg FFM^{-1} min^{-1})	Basal	2.85 ± 0.11	3.07 ± 0.28	2.99 ± 0.11	3.35 ± 0.36
	LI	1.52 ± 0.17	1.79 ± 0.20	1.62 ± 0.16	1.93 ± 0.17
	HI	1.11 ± 0.32	1.41 ± 0.21	1.96 ± 0.36§	2.03 ± 0.37
EGS (mg kg FFM^{-1} min^{-1})	Basal	0.51 ± 0.51	0.30 ± 0.47	0.46 ± 0.43	0.22 ± 0.61
	LI	0.92 ± 0.32	0.37 ± 0.38	0.92 ± 0.47	0.33 ± 0.37
	HI	7.17 ± 0.87	5.95 ± 0.73	6.49 ± 0.64	5.51 ± 0.65
LIPOX (mg kg FFM^{-1} min^{-1})	Basal	1.01 ± 0.11	1.20 ± 0.03	1.15 ± 0.06	1.15 ± 0.06
	LI	0.70 ± 0.07	1.16 ± 0.04****	0.81 ± 0.06	1.04 ± 0.04***
	HI	0.13 ± 0.06	0.78 ± 0.06****	0.11 ± 0.06	0.72 ± 0.01****
EE (kJ kg FFM^{-1} 24 h^{-1})	Basal	134.6 ± 2.6	132.0 ± 2.8	134.0 ± 3.8	134.0 ± 2.8
	LI	118.4 ± 6.7	136.1 ± 2.1****	129.3 ± 3.3	132.7 ± 2.5
	HI	137.6 ± 2.5	139.2 ± 2.6	138.1 ± 3.6	144.1 ± 3.7*

SAL vs LIP: *P < .05, **P < .01, ***P < .001, ****P < .0001; LBW vs NBW: §P < .05.

basal, LI, and HI: no change), lipid oxidation (LIPOX: basal, no change; LI and HI, ↑; P < .0001), and energy expenditure (EE: basal, no change) (Table 4). Whereas lipid infusion tended to increase EGP in the NBW group at LI (LI-LIP vs LI-SAL; P = .065), no such change was observed in the LBW group (LI-LIP vs LI-SAL, NS), and consistently with the findings during saline infusion, EGP tended to be lower in the LBW group at HI (HI-LIP, LBW vs NBW; P = .1). Energy expenditure increased significantly at LI in the LBW group (LI-LIP vs LI-SAL; P < .0001) but not in the NBW group, and increased at HI in the NBW group (HI-LIP vs HI-SAL; P < .05) but not in the LBW group. Finally, as during saline infusion, insulin-stimulated GF was lower in LBW subjects than in NBW controls (HI-LIP, LBW vs NBW; P < .05).

3.4. Intravenous glucose tolerance test

Both groups showed a similar increase in insulin/C-peptide concentrations and prehepatic ISRs in response to lipid infusion (Table 5). As a result, the disposition indices were similar between the groups (DI-LIP, LBW vs NBW, NS), and unaffected by intralipid infusion (LBW, DI-LIP vs DI-SAL, NS; NBW, DI-LIP vs DI-SAL, NS).

4. Discussion

We found that a 24-hour low-grade FFA exposure yielding FFA concentrations similar to what can be seen in type 2 diabetic patients (ie, marginally higher in the postabsorptive state but several-fold higher during [meals and] insulin stimulation) reduced whole-body insulin sensitivity similarly in young LBW subjects and matched NBW controls. Moreover, the FFA-induced decline in insulin sensitivity was met by an appropriately balanced, compensatory increase in glucose-stimulated insulin secretion (GSIS) in both groups, regardless of birth weight. The LBW subjects had significantly lower insulin-stimulated glycolysis after lipid infusion than the NBW controls. The significance of this observation remains to be determined but confirms findings previously reported by our group [28].

Table 5
Intravenous glucose tolerance test

	LBW		NBW	
	SAL	LIP	SAL	LIP
Si_{Rd}	0.0058 ± 0.0005	0.0045 ± 0.0006**	0.0064 ± 0.0005	0.0053 ± 0.0007*
Incremental $AUC_{glucose}$	82.9 ± 3.1	85.8 ± 2.1	80.2 ± 2.1	86.6 ± 2.5***
Incremental $AUC_{insulin}$	2376 ± 296	3021 ± 331***	2405 ± 244	3104 ± 316**
Incremental AUC_{ISR}	108.1 ± 10.8	124.4 ± 11.3**	103.4 ± 8.2	119.8 ± 10.7**
DI	0.6012 ± 0.0783	0.5361 ± 0.0844	0.6380 ± 0.0590	0.6018 ± 0.0946

SAL vs LIP: *P < .05, **P < .01, ***P < .001. LBW vs NBW, all NS. For units please see Subjects and Methods section.

4.1. Effect of FFA on glucose disposal and cellular glucose metabolism

Using the "gold standard" hyperinsulinemic-euglycemic clamp technique in combination with [3-^{3}H]-glucose, we found that a 24-hour low-grade intralipid infusion reduced whole-body insulin-stimulated glucose disposal by approximately 12% to 14% in 19-year-old healthy lean men, *regardless* of weight at birth. This rather modest effect was almost completely accounted for by reduced glucose oxidation, whereas glucose storage was not significantly altered by intralipid infusion. Few lipid infusion studies have been carried out in individuals at increased risk of diabetes. Insulin sensitivity (assessed by a hyperglycemic clamp) was similarly reduced, by ~40%, after a 48-hour 2-fold elevation of fasting plasma FFA in young obese nondiabetic Oji-Cree individuals and matched white controls [42]. Using a more physiological FFA dose, we previously reported that both glucose-intolerant middle-aged first-degree relatives of type 2 diabetic patients and matched obese controls experience a 25% to 30% reduction in peripheral glucose disposal after a 24-hour lipid infusion [43], whereas a similar elevation of plasma FFA (500-800 mmol L^{-1}) extended to 3 [44] or 4 [45] days caused an approximately 25% decrease in insulin-stimulated glucose disposal in 40-year-old control subjects but had no significant effect in glucose-tolerant insulin-resistant individuals with genetic predisposition to T2DM (both assessed by hyperinsulinemic-euglycemic clamp). Although comparison of the results is made difficult by different study designs, the majority of the studies including the present one do not support a heightened sensibility to elevated FFA at the site of the periphery in at-risk populations. However, it is worth noting that in contrast to our study population, most of these individuals are already insulin resistant and/or glucose intolerant, and a small further impairment of glucose uptake could potentially have a significant impact. Accordingly, although the intralipid challenge did not prove more deleterious to the LBW subjects, it may well be that a similar challenge imposed at a later time, in the presence of obesity or overt metabolic defects, would be more harmful.

4.2. Effect of FFA on glucose production

Significantly higher endogenous insulin release during clamps and, perhaps as a result thereof, no increase in glucagon concentrations during lipid infusion in the LBW group may have brought about or contributed to the beneficial effects on the liver. Better insulin suppression of the liver was previously shown in protein-restricted rats exposed to a high-fat diet for 8 weeks [27], and in glucose-intolerant first-degree relatives of type 2 diabetic patients after a 24-hour low-grade intralipid infusion [46], and may represent a compensatory mechanism to ensure better overall glucose tolerance.

4.3. Effect of FFA on insulin secretion

The effect of prolonged FFA elevation on GSIS in humans is controversial. Prolonged (24-48 hours) FFA elevation either increased [47] or decreased [42,48,49] GSIS in lean nondiabetic subjects. Moreover, it remains a topic of much debate whether individuals at increased risk of diabetes (obesity, family history, ethnicity) are more susceptible to the actions of elevated FFAs than matched controls. Normal glucose-tolerant and IGT family members of type 2 diabetic patients had reduced compensatory β-cell response to insulin resistance associated with obesity compared with matched controls at mean age 40 [50]. A 2-fold elevation of basal FFA levels for 48 hours significantly reduced GSIS, assessed by C-peptide deconvolution, in middle-aged obese nondiabetic individuals but *not* in patients with type 2 diabetes [51], or individuals from the Oji-Cree population [42], whereas 2 recent studies using a more physiological approach have yielded conflicting results: data from our group have suggested that *both* elderly obese first-degree relatives of type 2 diabetic patients with IGT *and* age- and BMI-matched family history-negative controls are incapable of increasing insulin secretion sufficiently to match the FFA-induced insulin resistance after a 24-hour low-grade intralipid infusion [43], whereas sustained infusion of FFA for 3 days markedly impaired insulin secretion in response to a mixed meal and glucose in 40-year-old lean family-history–positive individuals, with no apparent deleterious effect in matched controls [44]. The effect of prolonged lipid exposure on insulin secretion in LBW individuals had not been previously studied. We found that at age 19, lean glucose-tolerant men with birth weights below the 10th percentile were perfectly capable, as were their matched NBW controls, of increasing insulin secretion adequately in response to the FFA-induced insulin resistance (assessed by deconvolution of peripheral C-peptide concentrations). Interestingly, plasma C-peptide concentrations during clamp (reflecting endogenous insulin release) were significantly increased during intralipid infusion in the LBW group compared to controls. A role for insulin in regulating its own secretion, in an autocrine feedback manner, has long been a topic of debate. Whereas some studies show a negative feedback effect of insulin on its own secretion [52,53], others fail to support this concept [54,55], and yet others suggest a stimulatory effect of insulin at low concentrations [56]. Although we cannot completely rule out a carryover effect from the IVGTT, the slightly but significantly higher circulating C-peptide (and insulin) concentrations in the LBW group may be of significance and could potentially lead to increased fat storage, β-cell exhaustion, and/or induction of insulin resistance in the long run.

In conclusion, at age 19, prolonged exposure to physiological levels of plasma FFAs did not prove more disadvantageous to LBW men than matched NBW controls, at least not at the whole-body level. We have no way of

knowing whether our LBW cohort is representative of LBW cohorts in general, and we may not have sufficient power to detect small differences in these healthy lean men. Nonetheless, despite being perfectly matched with regard to BMI, W/H ratio, and total fat mass, we recently reported altered body composition and increased abdominal fat mass [57], as well as major reductions of several insulin-signaling proteins in skeletal muscle taken from these same LBW individuals [58]. These features are potentially diabetogenic and precede overt metabolic derangement. It remains to be determined whether FFAs exert differential effects at the tissue level, or whether factors other than elevated plasma FFA but related to increased fat mass may account for the apparent additive effect of an adverse early environment and obesity/high fat feeding observed in some animal and human studies. Additional studies are necessary to address these possibilities. The reproducibility of lower glycolysis in the LBW group during insulin infusion, combined with the fact that low birth weight has previously been associated with impaired glycolysis in humans using different methodology and study design [59], is interesting and merits further study. However, the significance of this finding remains to be determined and should be confirmed in other LBW populations as well.

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